Research Topics

Biodiversity across afromontane environments

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Biodiversity across afromontane environments

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Editorial: Biodiversity across Afromontane environments

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Editorial on the Research Topic
Biodiversity across Afromontane environments

Mountains have always fascinated humans. Their remoteness, grandiosity, stability, and wilderness awake in most of us admiration, which motivates us to climb their steep slopes again and again. The pure biological perspective is fascinating, too. Over relatively small spatial scales, montane elevational gradients support a variety of environments, thus leading to biogeographical patterns with an elevated diversity of species. This is especially true in the tropics, where stable climatic conditions across different scales of time reinforce the effects of geographical isolation and thus support the origin and maintenance of biological diversity. In Africa, temperate and sub-tropical mountains hold as much local diversity and endemism as do the tropical mountains, such as the exceptional plant diversity in the Cape Fold Mountains of South Africa and the rich local endemism in the Atlas Mountains in northern Africa.

In the global context, African mountains are unique, most of them being fragmented systems, with overall intra African mountain isolation being higher than on other continents where extensive linear fold mountains and volcanic chains are "typical." This fragmentation has given rise to unusual patterns of endemism and diversity that have been the subject of much debate and research. Still, our knowledge of mountains in Africa is very limited, with large swathes of mountain systems partly, patchily, or wholly unexplored by biodiversity scientists. In addition, African mountain ecosystems are significantly altered through direct human impact. Montane cloud forests are disappearing at high rates, vast areas had already disappeared decades ago, and the conversion of natural montane grassland to tree plantations has dramatically increased threats to local endemics among plants and animal groups. This is currently coupled with the potential effects of climatic change on African mountain systems, leading also to the disappearance of specialized glacier ecosystems on the mountain tops.

This Research Topic aims to emphasize that African mountains continue to offer invaluable biological insights and material for ecological and evolutionary thinking. This holds true even though tropical mountains have already been studied for two centuries since Alexander von Humboldt. After such a long period, we can still miss significant pieces of information to understand mountain biological diversity comprehensively; knowledge of African mountains can help significantly in this regard. Such an understanding is crucial not only for pure scientific insights but also for the effective conservation of Afromontane environments and their endemic inhabitants, which are rapidly disappearing. Thus, this Research Topic also endeavors to promote conservation efforts in montane environments across the African continent.

The spectrum of potential research questions related to African mountains is wide. At the time of opening this Research Topic's call, we encouraged the potential authors to submit contributions focused on a variety of ecological and evolutionary topics, from community ecology and biogeography to evolutionary drivers of biodiversity patterns, including multidisciplinary research linking ecology, evolution, ecophysiology, geography, meteorology, and geology. Nineteen manuscripts were submitted, of which 12 were accepted; despite this expected attrition, the accepted papers adequately covered the intended scope of the Issue. Biodiversity hotspots and endemic species are the golden thread through this Research Topic. Clark et al. show that even large areas in the relatively well-studied region of South Africa remain overlooked regarding endemic species. Seifert et al. reveal how biodiversity hotspots can be modified by humans. Specifically, they found that natural ecosystems and transitions biotopes in the Taita Hills of East Africa contribute positively to ecosystem functions, which contrasts with human-modified landscapes. Even though restricted-range species are endangered, and many species are predicted to decline in the future, some climatic predictions suggest an increase in the availability of suitable habitats, as reported for the Walia Ibex by Gebremedhin et al.. Forecasted climate change influence on Afrotropical forest species richness seems to be different in comparison with data from Neotropics, which hampers the generalizations (Núñez et al.). However, the global changes and their regional idiosyncrasies can be tracked in the mountain systems by observing the C3-C4 "grass line" as proposed by de Deus Vidal et al., a valuable contextual readjustment in montane systems without an observable treeline. Sharp Afrotropical elevational gradients also offer unique study systems to test classical ecological questions. Komposch et al. show that deadwood biomass is highest at mid-elevations of Mount Kilimanjaro, which is especially a result of climatic gradients and anthropogenic disturbances. Ratier Backes et al. detected the effects of soil properties, temperature gradients, and disturbance on species richness of plant communities but not functional diversity in Tenerife. Temperature gradient has been proven responsible by Barshep et al. for body mass changes in birds across elevations in the Nigerian savanna. Surprisingly, some topics related to general ecological issues, such as environmental seasonality, remain largely unexplored in Afromontane environments, thus the description provided by Dinesen et al. of how seasonal changes affect the composition of bird communities serves as a valuable insight. African mountains are of course perfect model systems for investigating the role of geographical isolation in the evolution of new species and the phylogenetic structure of ecological communities. This Research Topic contains three papers touching on this field. Cuypers et al. highlight the biogeographical importance of the Livingstone Mountains in Tanzania as a crossroad for small non-volant mammals. The mammal assemblages tend to be phylogenetically and functionally less closely related toward high elevations on Mount Kenya, but the opposite pattern was found in the Chyulu Hills (Onditi et al.). "African biogeography" was founded in the Eastern African mountains, which form "sky islands" in the "sea" of the lowland savanna; still, many species are shared across locations. As a part of this collection, Fjeldså and Bowie analyzed the phylogeography of avian lineages to describe the historical relationships between lowland and montane faunas and provide an overall picture of historical changes in the geographical distributions of birds in this biodiversity hotspot.

Conclusions

This Research Topic provides stimulating reading that enhances both the research value and biological value of Africa's mountains. Already, from this set of contributions, it is obvious that research effort is not equally distributed across the continent. Eastern African mountains have traditionally attracted more research than elsewhere on the continent. More recently, southern Africa has become a stronger contender, while the limited contributions from Western Africa may be due to generally fewer mountains and less topographical complexity. Northern Africa remains the least well-represented. Africa is a continent of dramatic environmental gradients and burning environmental issues. Detailed and extensive knowledge of how changing environments influence biodiversity is thus crucial for adequate and effective sustainable development, so that humanity and nature can co-exist in African mountains.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Quest for New Space for Restricted Range Mammals: The Case of the Endangered Walia Ibex

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Populations of large mammals have declined at alarming rates, especially in areas with intensified land use where species can only persist in small habitat fragments. To support conservation planning, we developed habitat suitability models for the Walia ibex (*Capra walie*), an endangered wild goat endemic to the Simen Mountains, Ethiopia. We calibrated several models that differ in statistical properties to estimate the spatial extent of suitable habitats of the Walia ibex in the Simen Mountains, as well as in other parts of the Ethiopian highlands to assess potentially suitable areas outside the current distribution range of the species. We further addressed the potential consequences of future climate change using a climate model with four emission scenarios. Model projections estimated the potential suitable habitat under current climate to 501–672 km^2 in Simen and 6,251–7,732 km^2 in other Ethiopian mountains. Under projected climate change by 2,080, the suitable habitat became larger in Simen but smaller in other parts of Ethiopia. The projected expansion in Simen is contrary to the general expectation of shrinking suitable habitats for high-elevation species under climate warming and may partly be due to the ruggedness of these particular mountains. The Walia ibex has a wide altitudinal range and is able to exploit very steep slopes, allowing it to track the expected vegetation shift to higher altitudes. However, this potential positive impact may not last long under continued climate warming, as the species will not have much more new space left to colonize. Our study indicates that the current distribution range can be substantially increased by reintroducing and/or translocating the species to other areas with suitable habitat. Indeed, to increase the viability and prospects for survival of this flagship species, we strongly recommend human-assisted reintroduction to other Ethiopian mountains. Emulating the successful reintroduction of the Alpine ibex that has spread from a single mountain in Italy to its historical ranges of the Alps in Europe might contribute to saving the Walia ibex from extinction.

Keywords: connectivity, corridors, endemic species, distribution range, habitat loss, habitat suitability modeling, reintroduction, translocation

INTRODUCTION

There is increasing evidence that anthropogenic land use change poses imminent threats to biodiversity globally (Leadley, 2010; Watson et al., 2014). Indeed, humans have greatly altered the distribution ranges of endangered species and directly impacted their survival (Thuiller et al., 2005). Degradation of ecosystem services goes hand in hand with species extinctions, declining species abundances, and widespread shifts in species distributions (Leadley, 2010). The current rate of global extinction in biodiversity due to human activities is orders of magnitude higher than natural extinction rates (Pimm et al., 1995). Natural rates are calculated as approximately 1.8 extinctions per year per million species, whereas anthropogenic rates during the last 500 years have reached 28–100 extinctions per year per million species (Barnosky et al., 2011).

While land use change was considered the main driver of terrestrial biodiversity loss during the twenteith century, climate change is increasingly becoming a threat (Leadley, 2010). Climate change has started to impose multiple impacts on species, such as demographic and phenological changes, species range shifts and range size changes (Pounds et al., 1999; Bellard et al., 2012). Many species are shifting their ranges toward higher elevations and latitudes (Lenoir et al., 2008; Lovari et al., 2020), but migration is not always possible, because suitable areas can be lacking or because habitat loss has disrupted connectivity (Early and Sax, 2011). Given current trends, climate change could surpass habitat destruction as the greatest global threat to biodiversity over the coming decades (Leadley, 2010). The populations of large mammals have declined at alarming rates, especially in areas of intense land use where species can only persist in small habitat fragments, and often inside protected areas (Morrison et al., 2007). However, even protected areas face strong pressures from increasing human populations (McNeely, 1994). Hence, ensuring the survival of large mammals in human-dominated landscapes is challenging (Morrison et al., 2007; Gordon, 2009).

Modeling the suitability of habitats by means of species distribution models (SDMs) has become increasingly important to understand species ecology and develop conservation strategies (Morrison et al., 2007; Lobo et al., 2010), particularly to address the impact of climate change on species distributions (Araújo et al., 2004). SDMs assess the relationships between species occurrence data and environmental predictors to evaluate the suitability of a given area for a target species (Guisan and Zimmermann, 2000), and allow for identifying suitable areas for species conservation management (Zielinski et al., 2006; Thuiller, 2007). Multiple statistical algorithms are used for modeling species distributions. The combination of multiple models (ensemble forecasting) for suitability projections are a means to visualize uncertainty, thus providing more robust conclusions for conservation planning (Thuiller et al., 2006; Araújo and New, 2007; Marmion et al., 2009; Meller et al., 2014).

The Walia ibex (*C. walie*) is an endangered (EN) species restricted to the montane, sub-afro-alpine and afro-alpine habitats of the Simen Mountains in northern Ethiopia. It inhabits rugged and steep terrain in a single conservation area, the Simen Mountains National Park. The park was established as a direct response to the high conservation significance of the species. Main threats resulting in population decline of the Walia ibex originated from habitat loss due to agricultural expansion, overgrazing, constructions, and occasional poaching (Galvin and Haller, 2008). Low genetic variability, with an effective population size (N_e) much smaller than the census population size (Gebremedhin et al., 2009), is an additional threat. Genetic data have documented a past population bottleneck, which combined with its single small distribution area has led to extremely low genetic diversity; in fact, one of the lowest ever recorded in any endangered mammal species (Gebremedhin et al., 2009). The Walia ibex has the smallest current distribution of all wild goat species (<100 km^2), but it had a much larger range, including mountains beyond the park boundary, in the past (**Figure 1**). However, anthropogenic factors pushed the population to the verge of extinction, with an estimated total population of 150 individuals in the early 1990's. Due to conservation measures, the population is recovering, and current estimates suggest a population size of >957 (Ejigu et al., 2017). Given this trend, the protected area may not have adequate suitable habitat to support a larger population in the future. Proposed conservation actions include the establishment of viable populations in other suitable areas, for instance by facilitating movements or through translocations to adjacent unoccupied habitats (Hirzel et al., 2002; IUCN, 2013). Such actions require the identification of suitable habitat area outside the current park boundaries (Seddon et al., 2007). SDMs allow for identifying such suitable areas, thereby supporting a detailed planning of translocations or assisted colonization of new habitats. Given that climate change will further modify the distribution of potentially suitable habitats in the future, such scenarios should additionally be taken into account in conservation management planning.

In this study, we analyzed and estimated the spatial extent of the suitable habitats of the Walia ibex in the Simen Mountains, and projected our models to other Ethiopian mountains to assess whether areas with potentially suitable bioclimatic conditions exist. Being a mountain specialist, we expect the ibex to be negatively affected by climate warming in all mountains due to shrinkage of its habitat. To test this hypothesis and in order to understand the spatiotemporal dynamics of suitable areas, we addressed the potential consequences of future climate change. We implemented three different strategies to improve the reliability of our species distribution predictions. Specifically, (i) when predicting models under present and projected future climate conditions, we excluded any territory where the models extrapolated; (ii) we accounted for imperfect detection of presences; and (iii) we measured habitat features that are indicative of an increase or decrease in total suitable areas under present and projected future conditions. Our results are discussed and evaluated in a conservation context.

MATERIALS AND METHODS

Study Area

The Simen Mountains National Park (with area of 412 km^2), situated in northern Ethiopia at 13°9′57″–13°19′58″ northern latitude and 37°54′48″–38°24′43″ eastern longitude, is the only refuge for the Walia ibex (**Figure 1**). The park is topographically diverse with an elevation ranging from 1,900 to 4,543 m above sea level (a.s.l.). Agriculture is the dominant land use system. The natural vegetation is divided into three main zones: the montane forest belt, the transitional ericaceous belt, and the uppermost afro-alpine belt (Puff and Nemomissa, 2005).

Occurrence Data

The Walia ibex inhabits altitudes between 2,700 and 4,300 m a.s.l. (Ejigu et al., 2015), but historical data indicate that the species has inhabited altitudes as low as 2,300 m (Nievergelt, 1981). Here, we followed (Nievergelt, 1981) and considered all areas above 2,300 m in the Simen and in other Ethiopian mountains (**Figure 1**) as our study area.

The extant range of the Walia ibex is well known, and is the subject of regular monitoring (Gebremedhin et al., 2009, 2010). We performed repeated surveys of the Walia ibex habitats through the whole range of the species from March to April 2011. We used binoculars and telescopes to spot the animal from a distance. Presence points were recorded at the spot an individual or a group of Walia ibex were seen or fecal samples were observed. Individuals or groups of Walia ibex that were seen within less than about 100 m distance were considered as the same group. In such a way, we documented 214 occurrence points of Walia ibex.

Predictor Variables

Nineteen bioclimatic candidate variables at the 30 arc-seconds resolution (about 1 km^2) were obtained from WorldClim (Hijmans et al., 2005). Slope and aspect were additionally derived from the 90 m resolution Shuttle Radar Topography Mission digital elevation model (SRTM DEM) (Jarvis et al., 2008). The elevation model was resampled by using the nearest neighbor resampling algorithm by means in Google Earth Engine (GEE) to the same resolution as the WordClim variables before derivation of slope and aspect. Aspect was kept as a continuous variable in the analyses. We used GlobCover (Arino et al., 2012), resampled to 1,000 m using the majority nearest neighbor resampling algorithm in GEE, to represent land cover. Each land cover class was converted to 0 and 1 by specifying each category data as a dummy variable.

To limit multicollinearity effects, we evaluated pairwise Pearson correlations among predictor variables, and selected only variables with $r < |0.7|$, selecting the ones with higher biological importance. We retained eight variables: slope angle, slope aspect, land cover, isothermality (BIO3), temperature annual range (BIO7), precipitation of the wettest month (BIO13), precipitation of the warmest month (BIO18) and precipitation of the coldest quarter (BIO19).

All models from the three algorithms were projected to all Ethiopian highlands (areas >2,300 m; **Figure 1**), both for current and projected future climates, using the same criteria as described above to assess suitability. We projected the models to future climates representing the year 2,080 using data from the Community Climate System Model version 4 (CCSM4) relying on all the four emission scenarios (RPCs 2.6, 4.5, 6.0, and 8.5 Wm^2 of radiative forcing).

Model Fitting and Evaluation

Three statistical modeling approaches were applied, differing in general performance and statistical properties: (1) The Maximum Entropy Model (MaxEnt), a parametric maximum likelihood approach (Phillips et al., 2006; Halvorsen, 2013; Halvorsen et al., 2015); (2) The Generalized Additive Model (GAM), a non-parametric maximum likelihood approach (Wood, 2011); and (3)

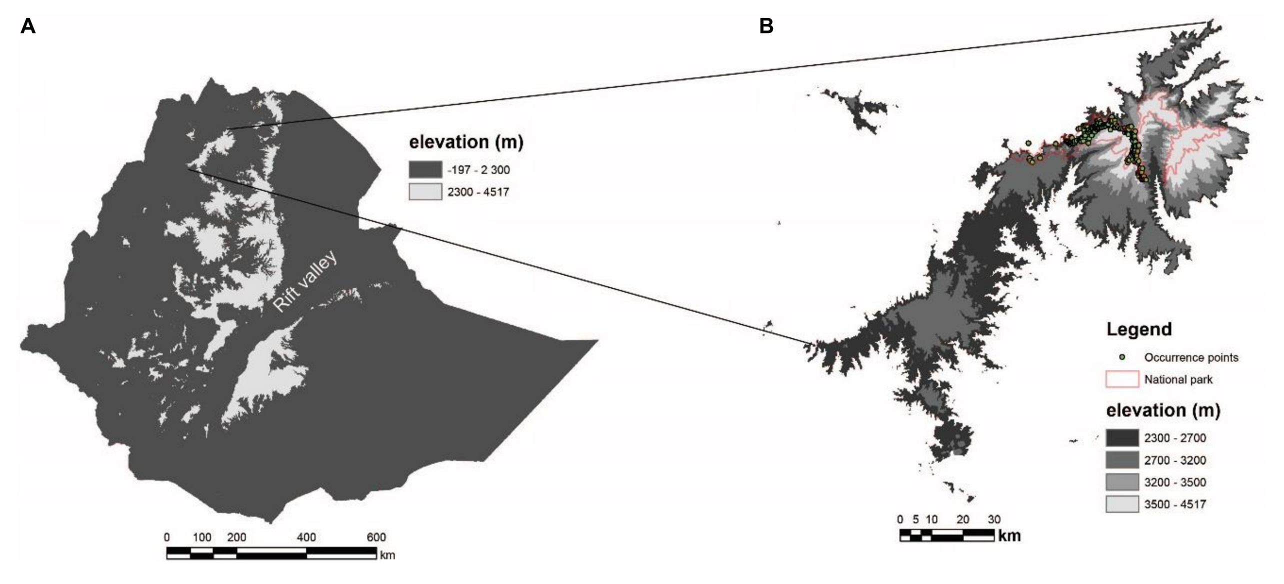

FIGURE 1 | Study area showing the Ethiopian highland masses above 2,300 m **(A)** and Simen Mountains **(B)**, the only place where the Walia ibex is extant today. The park boundary and the occurrence points used in the models are indicated in **(B)**.

The Gradient Boosting Model (GBM), a resampling (boosting) approach (Friedman et al., 2000; Elith et al., 2008).

The different modeling approaches calls for different way of creating absence points (Barbet-Massin et al., 2012). For MaxEnt and GAM we used the thickening procedure proposed by Vollering et al. (2019a) for collection of absences (background points and pseudo-absences). We generated 10,000 weighted points within discrete thickening distances by analyzing geostatistical properties of the predictors such as semi-variance. The weighting was performed by using the relative probability of absences to be equal to the number of presences within a radius length of each location determined of the spatial-autocorrelation range of the predictor variables (Vollering et al., 2019b). A minimum separation distance was set to 10 km. In the third algorithm (GBM), we randomly generated pseudo-absence points equivalent in number to the presence points that were collected during the field work. We then re-run the algorithm 10 times as recommended by Barbet-Massin et al. (2012), as tree-based machine learning algorithms requires this amount of runs at least when the number of presence points is less than 1,000 (Barbet-Massin et al., 2012).

Twenty-one replicates of MaxEnt models were fit with different regularization multiplier values (0–10 with an interval of 0.5) to regulate model complexity, using the DISMO package (Hijmans et al., 2013). We used the MIAmaxent R package for variable transformation and model selection for species distribution models. We finally retained the model with regularization multiplier of 3.5 due to the best cross-validation performance. We used the caret package in R to tune the parameters in the GAM models for choosing the optimal model across our parameters. Accuracy was used to select the optimal model using the largest value as criteria.

For the GBM approach, we built several models by setting the back fraction to the default value (0.75; Elith et al., 2008) and by using different combinations of tree complexity levels (1–5) and learning rates (slightly varying values ranging from 0.0001 to 0.05) from the "gbm.step" function in the gbm package (Ridgeway, 2013). From models with more than 1,000 trees, the least number of trees by default; see Elith et al. (2008), we selected the combination of tree complexity levels and learning rates that provided the lowest cross-validation deviance.

For each of the performed algorithms, we ran a fivefold cross-validation procedure and repeated it 10 times. To this end, we divided our data into five equal subsets, ran the cross-validation procedure, and repeated these steps 10 times. For each of the mentioned algorithms, we then calculated predictions from the weighted mean across all repeats and folds. All the three final models were validated against the test data set by means of the AUC and TSS accuracy metrics (Swets, 1988). The reported AUC of each algorithm is the average AUC computed across the repeated fivefold cross-validation. We also report TSS (true skill statistics) as an average across the repeated fivefold cross-validation. TSS was calculated as "sensitivity" + "specificity" – 1 (Allouche et al., 2006).

Spatial and Temporal Projections of Models

All models from the three algorithms were projected to all Ethiopian highlands (areas $>$ 2,300 m; **Figure 1**), both for current and projected future climates representing the year 2080 using data from the Community Climate System Model version 4 (CCSM4) by relying on all the four emission scenarios (RPCs 2.6, 4.5, 6.0, and 8.5 Wm^2 of radiative forcing).

The probabilistic prediction maps obtained from models runs as well as projections were split into binary presence-absence maps using three threshold criteria: (1) Minimum presence threshold, selecting the minimum probability value among occurrence points; (2) Maximum sum threshold (Maximum Sensitivity plus Specificity), maximizing the sum of sensitivity and specificity (Liu et al., 2005); and (3) Maximum Kappa, using the threshold that provides highest Cohen's Kappa values. All nine binary ensemble maps (three algorithms × three probability threshold criteria) were combined to define three habitat suitability classes: Unsuitable habitat, where less than 30% (maximum two out of nine) binary maps predict presence; Uncertain suitability, where 30–60% (3–5) maps predict presence; and Suitable habitat, where $>$60% (more than five) maps predict presence following Chala et al. (2016).

Future habitat suitability ensembles were produced by combining 36 maps (three algorithms, three threshold criteria and four emission scenarios) and produced the three habitat suitability classes following the same approach that was applied to the ensemble current habitat suitability map. We performed a MESS analyses (Elith et al., 2010) by using the R-package "dismo" (Hijmans et al., 2013). The MESS analyses show how far pixel values in the predictors are outside the range of presences in the training data. Negative values indicate that one or more predictors are outside this range and the MESS values decreases in accordance with the dissimilarity. Thus pixels with negative MESS values were ruled out and not included in the results.

Correction for Lack of Species Occurrences in Steep Areas

Ibex species are specialized to steep cliffs up to 60° (Nievergelt, 1981), which are difficult to access for predators and other organisms (Shackelton, 1997). The steepest areas ($>$45°) are, however, not likely to be well covered by species occurrence data obtained through visual observation, as in our case. Thus, we assumed that our presence-based models mainly captured the climate requirement of Walia, without fully capturing the topographic requirements, due to under-sampling of the steepest slopes. In order to take into account this source of bias, we repeated the whole analyses such that occurrences of Walia ibex in gentle slopes far away from steep cliffs were removed from the presence data set. We also repeated the whole analyses and built models without slope as predictor. We compared the two model versions, and filtered flat areas from both. To filter flat areas far away from steep cliffs, we calculated the average slope among occurrence points. Then we flagged all occurrence points for which the slope was lower than the mean value and calculated the distance between these points and the nearest cliff with slope above the mean. We computed the first (lower) quartile, the third (upper) quartile, and the interquartile range (IQR). In this way, we restricted the presence points for the Walia ibex to include only suitable habitats that overlap with the cliffs that are above mean values of the slope angle and any suitable habitat within the distance of the sum of the upper quartile value plus 1.5 times the IQR from these cliffs. Overall, for both current and future climate conditions we present two versions of habitat suitability maps for the Simen Mountains and other Ethiopian mountains, i.e., maps with and without steep area correction.

Landscape Connectivity

We performed a landscape connectivity analyses in order to provide an estimation of the habitat that can be spontaneously colonized by the Walia ibex under present and projected future climate conditions. Thus, we calculated two different connectivity indices in the program package FRAGSTATS (McGarigal, 2002): the proximity index and the connectance index. This analysis was limited to suitable habitats inside a "landscape" of 100 × 100 km with the current occurrence points of Walia ibex in the center, because available data on species distribution and movements do not support the idea of spontaneous dispersal over broader scales. Other typologies of connectivity analyses were hampered by limited data on species movements and lack of high-resolution information on fine-scale landscape elements that could act as barriers.

In this analysis, suitable habitats are defined as the habitats currently used by the Walia ibex. The proximity index is calculated between a given habitat spot (patch) and all other habitat spots of the same type. The sum of the area of the habitat spots (m^2) is divided by the square of nearest edge-to-edge distance (m^2). Therefore, the proximity index has higher values at increasing amounts of suitable areas and if suitable areas are nearby. The connectance index is the number of connections (c) between all habitat spots of the same type (i) (sum of cijk, where cijk = 0 if spot j and k are not within the specified distance from each other, and cijk = 1 if spot j and k are within the landscape, divided by the total number of possible connections between all spots of the same type, multiplied by 100 to give a percentage value (values between 0 and 100).

RESULTS

Predictions Under Current Climate

The three statistical models showed similarly high performance, with average AUC and TSS values from all model runs per algorithm: AUC = 0.902 and TSS = 0.846 for GAM, AUC = 0.905 and TSS = 0.853 for GBM, and AUC = 0.906 and TSS = 0.855 for MaxEnt. The predicted suitability patterns were also similar for the models (**Supplementary Figures 1**, **2**). Our results consistently showed that suitable habitat for the Walia ibex is available in the Simen Mountains, also in areas where the species is not currently known to exist. The model projections suggested that there is 501–672 km^2 of suitable habitat in the Simen Mountains (**Figure 2** and **Table 1**), considerably larger than the currently delineated protected area (**Figure 1**).

Projecting the models to all Ethiopian highland areas suggested that suitable habitat is not only present in the Simen mountains, but also in the several other mountain fragments in northwestern Ethiopia (**Supplementary Figure 2**). The GAM model also predicted suitable habitats in the southern highlands of Ethiopia, in the Bale and Arsi Mountains, southeast of the Rift Valley (**Supplementary Figure 2**). In the other two models, this was only the case when the minimum presence threshold was used. The models without slope as predictor resulted in reduced suitable habitat area, namely a 21.4% reduction in the Simen Mountains and a 20.4% reduction across all Ethiopian highlands (**Table 1**). The impact of under-sampling of the steepest slopes was lower in the Simen Mountains (−13.6%) than in other Ethiopian mountains (−28.4%; **Table 1**).

Overall, the modeling approaches indicate that there are fragmented patches with potentially high habitat suitability, mostly in the central northern highlands, west of the Rift Valley, and that most of these patches are isolated (**Figure 3** and **Supplementary Figure 2**).

Predictions Under Projected Climate Change

Model projections under future emission scenarios indicated a tendency of shifting suitable habitats toward higher altitudes, both in the Simen and in the other Ethiopian mountains (**Supplementary Figure 1**). Upward range shifts resulted in an expansion ranging from 501–672 to 1,164–1,243 km^2 of suitable habitat in Simen, depending on model and scenario applied. The opposite pattern was detected in other Ethiopian mountains (a reduction ranging from 6,251–7,732 to 4,922–5,812 km^2), with consistent results across models. Correction for under-sampling of the steepest slopes had less pronounced impact under climate change scenarios, especially in mountain areas outside Simen, reflecting that less flat area is found to be available or suitable toward mountain summits (**Table 1**). The MESS analyses showed four mountain areas outside Simen where our predictions may be unreliable (**Figure 4**). One of these areas was in the northern part of Ethiopia close to Dessie and three other areas in the South. The prediction cells in these territories were ruled out and excluded from the reported results.

Habitat Connectivity

The proximity analysis identified that several suitable areas are well connected to currently occupied patches (**Figure 5**). Under scenarios of climate change, some increase of connectivity levels are expected, with an increase up to 5.86%, particularly in the central Simen area. This is likely caused by the prediction of more future suitable habitats in this area. Slight increases of the proximity index under climate change may suggest that larger suitable patches in areas with high elevation would become better

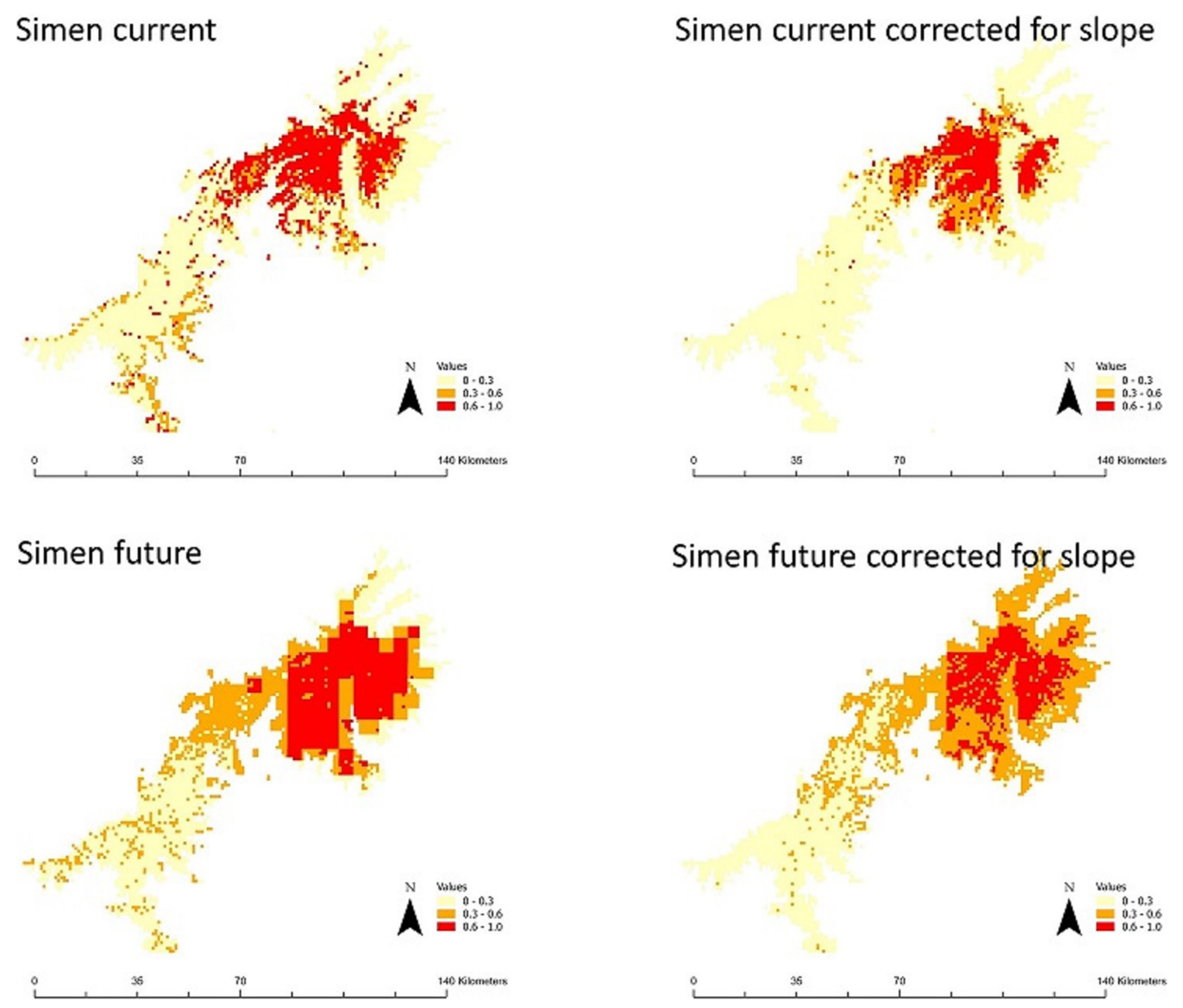

FIGURE 2 | Comparison of habitat suitability under current and future climate in the Simen Mountains. Current suitability maps are produced by averaging nine maps (3 algorithms × 3 threshold values). Future habitat suitability is produced by averaging 36 (3 algorithms × 3 threshold values × 1 climate model × 4 emission scenarios). The numbers on the legend bar refer to the degree of agreement among these maps in predicting presence: 0–0.3 unsuitable with high certainty, 0.3–0.6 uncertain suitability, >0.6 suitable with high certainty. Slope is excluded as a predictor variable and correction is made for under-sampling of steep slopes in the maps on the right panels.

TABLE 1 | The total area (km^2) of suitable habitat predicted with high certainty (>60% agreement for presence among models) in the Simen Mountains and among all Ethiopian highland masses (above 2,300 m), and correction for under-sampling of the steepest slopes.

	Simen mountains				**Whole Ethiopia**			
	With slope		**Without slope**		**With slope**		**Without slope**	
	Current	**Future**	**Current**	**Future**	**Current**	**Future**	**Current**	**Future**
Suitable habitat	738	1,418	580	1,345	10,970	6,626	8,731	7,100
Suitable habitat*	672	1,243	501	1,164	7,732	4,922	6,251	5,812

Area of suitable habitat corrected for under sampling of the steepest slopes.

connected in the future. In the opposite direction, connectance tended to decrease (**Table 2**), suggesting increasing distances between the total of patches, where the ibex at lower elevation would have to traverse larger areas of less suitable habitat.

DISCUSSION

This study is the first to examine both the current and future potential suitable habitats of the charismatic Walia ibex for all Ethiopian highlands, assessing in particular the expected impacts of climate change on habitat suitability. Our findings have important bearings on conservation management under changing climate and land use in Ethiopia. Indeed, our results demonstrate that there is suitable habitat available in the Simen Mountains and also in other Ethiopian mountains that is not yet realized, both under current and projected future climates. The particularly high suitability within the Simen Mountains confirms the significance of the area as a refuge for this endangered mountain mammal. All projected areas of high habitat suitability are located west of the Ethiopian Rift Valley, while suitability is generally lower on the eastern side (**Figure 3**). The projected area of suitable habitat varies depending on the predictors used in the model calibration, as models without slope tended to predict smaller areas of suitable habitat (**Table 1**).

Notably, our projections under climate change scenarios for the year 2080 suggest the possibility of an increase in the area of suitable habitat within the Simen Mountains, the only area where the species occurs today. In contrast, we found the opposite trend for all other parts of the Ethiopian highlands (**Table 1**). The projected expansion in the Simen Mountains is contrary to the general expectation of a shrinking of suitable areas for high-elevation species in response to climate warming and may be attributed to the ruggedness of the mountains, as has also been suggested in other areas of the world (Elsen and Tingley, 2015). The wide altitudinal range of the Walia ibex, and its ability to exploit the steepest slopes, might allow the species to track the expected vegetation shift to higher altitudes. The Simen is a complex mountain range with rugged topography presenting steep slopes compared to other Ethiopian mountains. As a consequence, the ibex could find additional, currently unexploited habitats under climate change (see **Supplementary Figure 1**). However, the potentially positive impact in Simen may not last very long under continued climate warming after

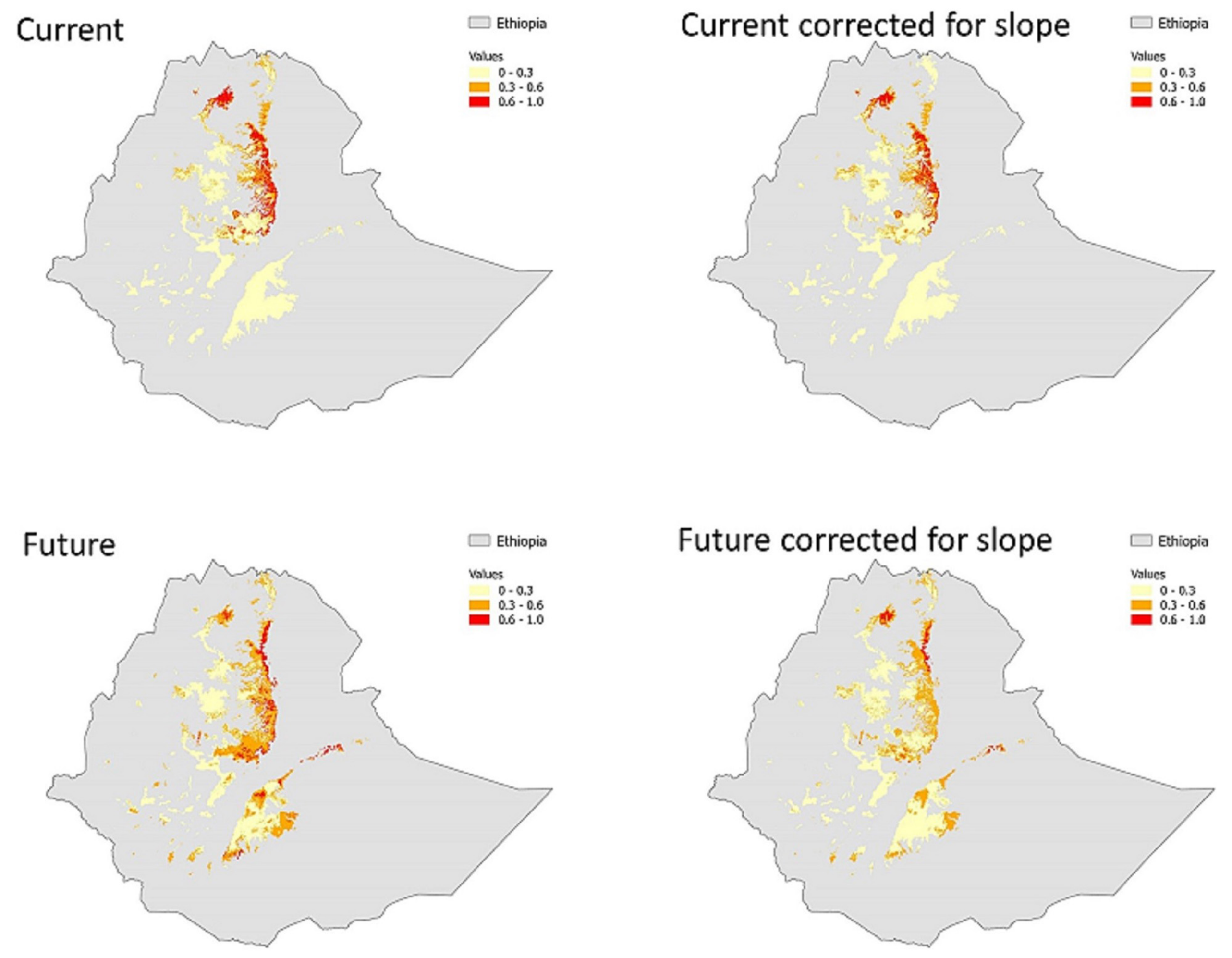

FIGURE 3 | Comparison of habitat suitability under current **(upper panels)** and future **(lower panels)** climates in Ethiopian highland masses (>2,300 m). Current suitability maps are produced by average nine maps (3 algorithms × 3 threshold values). Future habitat suitability is produced by averaging 72 maps (3 algorithms × 3 threshold values × 1 climate model × 4 emission scenarios). The numbers in the legend bar refer to the degree of agreement among maps in predicting presence: 0–0.3 unsuitable with high certainty, 0.3–0.6 uncertain suitability, >0.6 suitable with high certainty. Slope is excluded as a predictor variable and correction is made for under-sampling of steep slopes in the maps on the right panels.

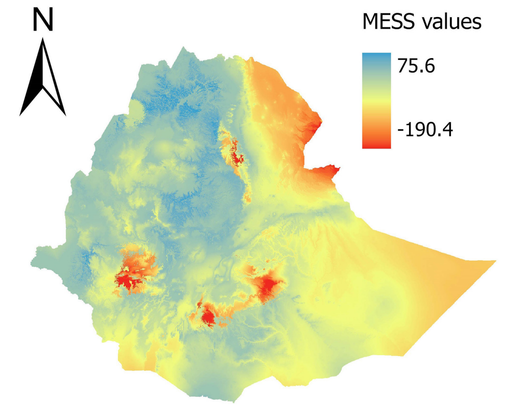

FIGURE 4 | MESS analysis. Negative MESS values showed by red colors have one or more predictors outside the range of present in the training data. Deeper red colors show larger differences and predictions here should be treated with strong caution.

2080, as the species will not have much more new space left to colonize.

A study on the impact of climate change on the gelada baboon (*Theropithecus gelada)* suggests that baboons could shift their lower altitudinal limit (>2,000 m) upward by about 500 m for every 2°C of increase in mean annual temperature, resulting in a loss of up to 32% in range size (Dunbar, 1998). Such a shift is primarily driven by the dietary behavior of this primate, which mainly feeds on grasses (Dunbar and Bose, 1991). The niche of the gelada baboon partially overlaps with the Walia ibex (Nievergelt, 1981) and it is documented that the Walia ibex previously could have inhabited lower altitudes, as low as 2,300 m a.s.l. (Nievergelt, 1981). In our surveys, however, tracks of the species were never observed below 2,700 m, which could imply that a shift toward higher elevations has already started. It is difficult to determine whether such a shift was caused by climate change or by direct anthropogenic impact. However, in the last few decades, agricultural expansion at low altitudes has been intensive, suggesting that habitat loss and/or competition with domestic goats (Gebremedhin et al., 2016) could have forced the ibex to move upwards. In our study, we did not include projections of land-use change, given the lack of high-resolution projections for the study area. However, regional analyses suggest that agricultural increase could cause further habitat shrinkage in the future (Williams et al., 2021), thus the prevention of habitat loss from agriculture is a conservation priority for the next years.

IUCN's guidelines for planning and managing protected areas recommend that protected areas (PAs) should be large and continuous with limited edge effects and with high connectivity to other areas of suitable habitat (Hamilton and McMillan, 2004). The Simen Mountains National Park satisfies none of these criteria, as it has been affected by considerable human intervention (Hurni et al., 2010). In addition to direct

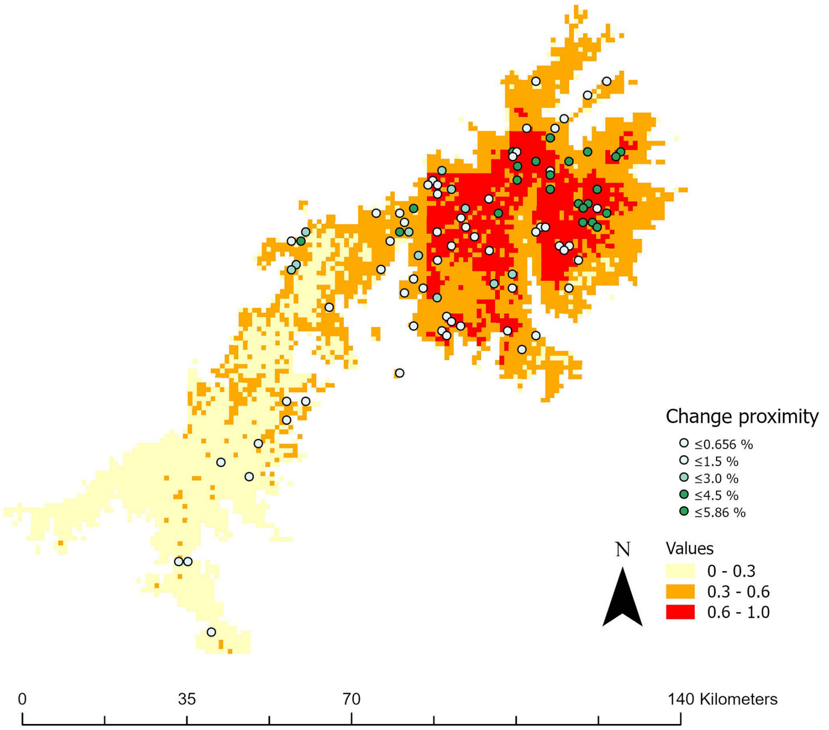

FIGURE 5 | Landscape proximity between present and suitable habitats for Walia ibex. The raster colors indicate the present-day proximity values. The dots represent the centroid of each suitable habitat patch. The color of the dots show how much the proximity value in each suitable patch would change (in percent) in the Simen mountain according to the prediction model Simen future corrected for slope showed in **Figure 2** lower right.

TABLE 2 | Average values for Proximity index and Connectance index for suitable habitat patches in the Simen Mountains, respectively, under current climate and future projection.

	Proximity	Connectance
Simen current	22.0695	7.8886
Simen future projection	23.2189	7.2981

anthropogenic factors, we show that climate warming will force the species to move further upwards. Under these circumstances, the pressure on small-sized protected areas such as the Simen Mountains National Park will become extremely high. Given the recent increase in population size of the Walia ibex, animals may already soon have to expand beyond the protected areas (Mason et al., 2014). The combination of anthropogenic factors, competition with livestock, and climate change may lead to further changes in the species' range dynamics that may render the small-sized currently protected area of insufficient quality in future conservation strategies and prompt the need for expansion of such areas. Indeed, future consideration of areas for reintroduction or translocation should focus on those that are well connected with other afro-alpine, sub-afro-alpine and montane ecosystems.

In response to the increasing human pressure on the habitats of the Walia ibex and the overall afro-alpine biodiversity, the total area of the Simen Mountains National Park was recently expanded from ∼225 to 412 km^2. With the enlargement of the protected area, the boundaries were redesigned to connect the currently occupied areas to the historical species distribution. So far, no animal movement to the reconnected areas has been observed. Our connectivity analysis suggests that proximity values between the Simen and some nearby suitable areas are not low, thus the expansion to nearby areas is in principle possible (**Figure 5**). However, our connectivity analyses focused on broad-scale suitability values, and did not take into account fine-scale landscape elements, such as barriers, that could actually hamper the movements of ibexes. More accurate assessments of potential colonization pathways would require high-resolution data on the occurrence of barriers, and better information on the actual movements of ibexes, for example through radiotracking. Such data would allow to identify areas where additional corridors and protected areas could be defined in order to increasing the chance for spontaneous re-colonization of historical sites.

Our study has identified potential key sites that can support re-established populations of the Walia ibex outside its current distribution range and has pointed to areas in which the species will also find suitable habitats under projected future climates. Planning and implementing conservation actions like reintroducing and translocating animals to other mountain areas have been successful for several close relatives of the Walia ibex, such as e.g., the Alpine ibex, *C. ibex* (Maudet et al., 2002). The Alpine ibex, endemic to the Alps in Europe, was driven very close to extinction in the early nineteenth century, when less than 100 individuals were estimated to be left in a single protected area in Italy (Stüwe and Nievergelt, 1991). The reintroduction to multiple sites within the historical range of the Alpine ibex in 1911 allowed populations to grow and to colonize many areas across all of the Alps (Hirzel et al., 2002). Such efforts are needed in order to conserve the Walia ibex in the highlands of the Ethiopian mountains.

Reintroductions are complex management actions that can have impacts also on the species that currently are resident in the target areas. Hence, it is important to consider the possible interactions between translocated species and the resident ones. Interspecific competition is one of the major potential issues. In a study conducted to assess the spatial overlap between reintroduced Bison (*Bison bison*) and resident ungulates, Jung et al. (2015) conclude that the overall potential for competition between reintroduced bison and resident ungulates is low, still they highlight that competition could occur across multiple niche dimension axes. A study on seven species of herbivores in the Simen Mountains suggested limited levels of competition (Dunbar, 1978). This could be because several of the species were in the process of re—establishing themselves following periods of absence or reduced density, so that competition was more likely to be incipient rather than actual.

The potential wild competitors for the Walia ibex in the Simen Mountains include the gelada baboon and the klipspringer (*Oreotragus oreotragus*) (Nievergelt, 1981). However, the density of klipspringer in the park is very low and the competition that may arise should not significantly affect the Walia ibex population (Dunbar, 1978). On the other hand, the density of domestic livestock in the study area has increased to a higher level over the last four decades (Gebremedhin et al., 2016). Studies performed on related species in India (Bagchi et al., 2004) observed very strong interactions between the Himalayan ibex (*C. sibirica*) and livestock species, suggesting that domestic livestock deplete the density and diversity of wild herbivores in the cold deserts of the Trans—Himalaya by imposing resource limitations. Similarly, using metabarcoding methodology Gebremedhin et al. (2016) documented potential competition with domestic goats in the Simen mountains. Even though reintroductions have a great potential to allow the Walia ibex regaining its historical range, accurate analyses of competition with both native and domestic species will be pivotal for the success of conservation actions.

CONCLUSION

The population size of the Walia ibex recently increased from 150 individuals in the early 1990s to a minimum of 957 individuals at present. Although the population increase is good news for the conservation of this iconic species, new tasks are ahead. The area currently protected by the National Park is very small, potentially leading to fierce competition for resources within the population as well as with the increasing number of domestic animals (Gebremedhin et al., 2016). Given the projected habitat suitability under climate change, new areas need to be considered for expanding the distribution of the Walia ibex. If appropriate management actions are undertaken, such as well-planned translocations and the building of suitable migration corridors (Stüwe and Nievergelt, 1991; Clark et al., 2002; Hirzel et al., 2002), the species could expand its population further, especially in the Simen Mountains, but potentially also in other areas west of the Ethiopian Rift Valley. A future presence of the Walia ibex in several interconnected mountain fragments that ideally form a functional metapopulation would certainly increase the viability and future prospects of this charismatic flagship species. The historically successful strategy of reintroduction of the once-nearly extinct species of the Alpine ibex that has roamed to the whole Alps mountains should be taken as a lesson to enable the Walia ibex to occupy suitable habitat in the Simen and other mountains west of the Rift Valley.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study didn't require animal handling. The field survey and data collection were conducted without disturbing the animals.

AUTHOR CONTRIBUTIONS

BG collected the data in the field. DC conducted the data analyses with input from VB, NZ, and GF. BG and ØF drafted the manuscript and was improved and revised by all co-authors. All authors contributed to the intellectual conception and design of the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.611632/full#supplementary-material

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Effects of Soil Properties, Temperature and Disturbance on Diversity and Functional Composition of Plant Communities Along a Steep Elevational Gradient on Tenerife

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Elevational variation of vegetation has been of interest for centuries, and a prominent example for such pronounced vegetation changes can be found along the steep elevational gradient on Tenerife, Canary Islands, 200 km off the West-African cost. The 3,718-m ascent to the peak of the island volcano, Teide, offers a unique opportunity to investigate associated changes in vegetation. However, elevation is not a directly acting factor, but represents several natural environmental gradients. While the elevational variation of temperature is globally rather uniform and temperature effects on plant communities are well understood, much less is known about the region-specific elevational change of chemical soil properties and their impact on plant communities along elevational gradients. Because human interference takes place even at high-elevation areas, we considered human-induced disturbance as important third factor acting upon plant community assemblages. In our study, we compared the effects of soil properties, temperature and disturbance on species richness, functional identity and functional diversity of plant communities along the elevational gradient on Tenerife. We used pairs of study plots: directly adjacent to a road and in natural vegetation close by. In each plot, we did vegetation relevées, took soil samples, and installed temperature loggers. Additionally, we collected leaf samples to measure leaf functional traits of 80% of the recorded species. With increasing elevation, soil cation concentrations, cation exchange capacity (CEC) and pH decreased significantly, while the soil carbon to phosphorus ratio slightly peaked at mid-elevations. Temperature had the strongest effects, increasing species richness and favoring communities with fast resource acquisition. Species richness was higher at road verges, indicating the positive effect of reduced competition and artificially generated heterogeneity. However, we did not detect road effects on plant functional characteristics. Vice versa, we did not find soil effects on species richness, but increased concentrations of soil cations favored acquisitive communities. Surprisingly, we could not reveal any influence on community

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functional diversity. The importance of temperature aligns with findings from large-scale biogeographic studies. However, our results also emphasize that it is necessary to consider the effects of local abiotic drivers, like soil properties and disturbance, to understand variation in plant communities.

Keywords: species richness, mountains, climate gradient, altitudinal gradient, chemical soil properties, road disturbance, environmental filtering, plant functional traits

INTRODUCTION

Plant community changes and plant species distributions along elevational gradients are a century-old topic in biogeography (e.g., von Humboldt, 1849; Whittaker and Niering, 1965). A prominent example of a pronounced vegetation sequence described by Alexander von Humboldt (von Humboldt and Bonpland, 1831) occurs along the steep elevational gradient on the island of Tenerife, Canary Islands, 200 km west of the African coast. Along the slopes of Mount Teide, vegetation belts comprise coastal and thermo-sclerophyllous scrub at low elevations, forests of Canary Island pine (*Pinus canariensis* C. Sm.) at mid-elevations and alpine scrub within the high-elevation volcanic caldera (Fernández-Palacios, 1992; **Figure 1**). In addition to such elevational variation in taxonomic composition, elevational patterns of plant species richness were observed worldwide (e.g., McCain and Grytnes, 2010; Haider et al., 2018). More recently, studies also addressed how community functional identity and functional diversity vary along elevational gradients (Schellenberger Costa et al., 2017; Midolo et al., 2021).

Elevation *per se* is not directly acting upon vegetation, but represents a number of environmental conditions (Körner, 2007). Two major environmental components changing with elevation and relevant for shaping plant communities are temperature and chemical soil properties. Temperature decreases with increasing elevation in mountains worldwide and adiabatic lapse rates have been intensely studied (Barry, 2008). In contrast, elevational changes of chemical soil properties are less frequently studied, region-specific and depend on multiple factors, such as bedrock, soil age and climatic conditions (Köhler et al., 2006; Dinter et al., 2020). These factors may affect soil acidity as well as biochemical cycles which can in turn modify concentrations of plant available nutrients (Grieve et al., 1990; Köhler et al., 2006; Unger et al., 2012; Tan and Wang, 2016). Since the environmental conditions shaping chemical soil properties (e.g., precipitation) do not necessarily change linearly with elevation (Körner, 2007), also the change of chemical soil properties might not be linearly correlated with elevation.

Soil properties are often modified by human actions (Weil and Brady, 2016). In mountains, especially the building of road infrastructure, road maintenance activities and disturbance through vehicle traffic and trampling can cause severe physical and chemical changes of road verge soil (Forman and Alexander, 1998; Johnston and Johnston, 2004). This might include higher concentrations of nutrients and less acidic soil pH, for example as a consequence of road dust, and moister conditions from road drainage (Forman and Alexander, 1998; Müllerová et al., 2011). Vehicles accessing the road verge may also lead to higher bulk density of the soil, causing lower soil permeability and thus reduced water and nutrient flows (Johnston and Johnston, 2004; Makineci et al., 2007; Deljouei et al., 2018; Yang et al., 2018). Therefore, even though soil nutrient concentrations might not differ between road verges and more natural vegetation away from the road, nutrient availability for plants may be lower in close proximity to roads as the roots may experience difficulties in penetrating hard soil or oxygen stress due to less aeration (Stirzaker et al., 1996; Makineci et al., 2007).

Both warmer temperatures and better soil conditions (more nutrients and neutral pH) have been found to increase the number of species in plant communities (Laughlin et al., 2007; Liu et al., 2020). Road disturbance should reduce competition among plants, and thus also enable a larger number of species to co-exist (Catford et al., 2012; Milbau et al., 2013; Haider et al., 2018). Moreover, temperature and soil conditions have been recognized to modify the functional characteristics of plant communities (Schellenberger Costa et al., 2017). So-called plant functional traits link morphological and physiological features to a plants' performance (Violle et al., 2007). Functional traits measured at the individual level can be translated to the level of species assemblages via abundance-weighted community trait means and community functional diversity. Trade-offs between traits associated with resource acquisition vs. resource investment define opposing ecological strategies observed in plant species and communities. Hence, functional traits are suitable proxies to describe the competitive ability of species and the competition intensity within a community, respectively. Acquisitive strategies are characterized by fast nutrient uptake and fast growth of short-lived leaves, and therefore characteristic for species with high competitive ability, represented e.g., by high values for specific leaf area (SLA) and leaf nitrogen concentration. Vice versa, conservative strategies are related to slow nutrient uptake and slow growth of highly durable leaves, and therefore characteristic for species with high competitive ability, represented e.g., by high values for leaf dry matter content (LDMC) and high amounts of carbon in the leaves (Wright et al., 2004; Díaz et al., 2016).

Many studies have shown community leaf traits to shift from acquisitive to conservative strategies with increasing harshness of environmental conditions, such as temperature (Moles et al., 2014; Rosbakh et al., 2015; Bruelheide et al., 2018). However, fewer studies investigated functional trait responses at the community level to varying soil conditions, especially along elevational gradients (but see Gong et al., 2020; Midolo et al., 2021). Paralleling the effect of benign climatic conditions, better soil conditions with higher nutrient concentrations and intermediate pH should lead to more acquisitive plant communities, e.g., with higher SLA (Molina-Venegas et al., 2018)

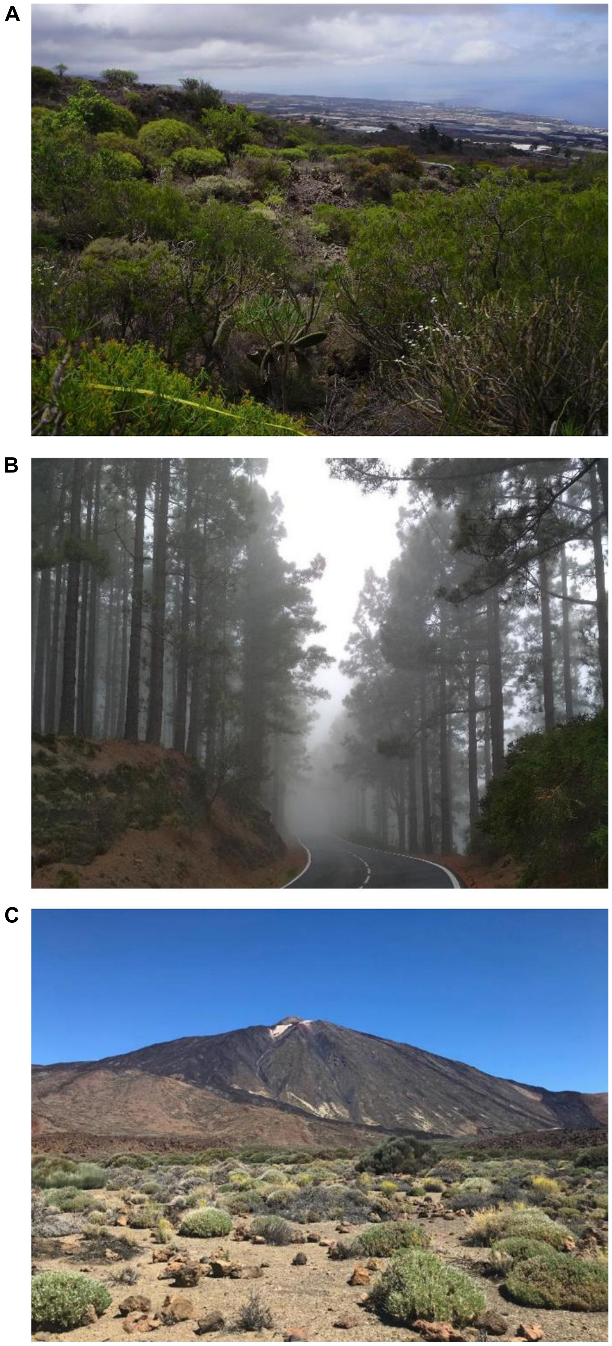

FIGURE 1 | The elevational gradient sampled in Tenerife starts with **(A)** coastal vegetation and thermo-sclerophyllous scrub, followed by **(B)** forests of Canary Island pine (*Pinus canariensis* C. Sm.), and ending at the high elevation plateau of Las Cañadas with **(C)** high mountain scrub.

and higher concentrations of leaf nitrogen (Schellenberger Costa et al., 2017). In contrast, it was found that plant communities on nutrient-poor soil had lower leaf nutrient concentrations as well as lower SLA (Wright et al., 2017). Additionally, by increasing the availability of resources (space, light, nutrients), road disturbance also favors species and communities with a more acquisitive strategy (Forman and Alexander, 1998). An increase in SLA and a decrease of LDMC were observed at sites with experimental physical disturbance (Bernhardt-Römermann et al., 2011), and an increase in leaf nitrogen was found at high disturbance intensity (Carreño-Rocabado et al., 2012).

Environmental factors like temperature and chemical soil properties do not only affect community trait means, but also community functional diversity. Environmental filtering is assumed to constrain species traits to certain values (Keddy, 1992), resulting in a decrease of functional diversity under harsh environmental conditions, e.g., in areas where the climate is characterized by low temperatures (Jiang et al., 2018) or where the soil is very poor in nutrients or has extreme pH values. Studies of functional diversity along soil nutrient availability are scarce (but see Schellenberger Costa et al., 2017; López-Angulo et al., 2020; Midolo et al., 2021). Another important assembly mechanism driving the functional diversity of plant communities is the concept of limiting similarity (Macarthur and Levins, 1967; Mouillot et al., 2007). In this case, functional differences between co-existing species reduce competition, resulting in communities with high functional diversity (Mouillot et al., 2007). Another factor that comes into play is road disturbance. The removal of plant biomass and consequently of dominant competitors as part of road maintenance will allow the entry of more individuals into the community as competition for space, light and nutrients decreases. With the addition of more individuals, functional diversity should increase due to a pure sampling effect (Mayfield et al., 2010).

In our study, we investigated how environmental conditions modify plant community characteristics along a gradient on Tenerife spanning more than 2,300 m in elevation. Thereby, we asked: (1) How do soil properties change along the elevational gradient and how do soil properties differ between road verges and away-from road locations? (2) Which environmental factors (temperature, soil properties, and road disturbance) have the strongest effects on species richness, functional identity, and functional diversity of plant communities?

MATERIALS AND METHODS

Study Area

Our study was carried out on Tenerife, the largest of the Canary Islands, situated in the mid-Atlantic between 27° 38′ and 29° 25′ off the West-African coast. The highest point on the island is the summit of the volcano Mount Teide at 3,718 m a.s.l. The crossover of different weather system causes the humid air masses of the north-eastern trade winds to remain on the northern slopes. Consequently, the southern slopes of Tenerife, where this study's elevational gradients are located, are characterized by an arid and warm climate (Fernández-Palacios, 1992). Temperature decreases along the elevational gradient with a lapse rate of approximately 0.5°C per 100 m (Morales Matos and Pérez González, 2000). Precipitation increases from approximately 100 mm per year at the coast to a maximum of approximately 500 mm at mid-elevations and moderately decreases again toward the summit, but with high variability due to the island's complex topography (Morales Matos and Pérez González, 2000; AEMET, 2012). This characteristic distribution of precipitation and temperature, the history of volcanic activity, and the specific topography of the island, lead to the formation of many different types of soil (Morales Matos and Pérez González, 2000).

Study Design

Data collection took place in April and May 2018. All data and samples were retrieved from the permanent plots which were established in 2008 following the design of the standardized mountain road survey of the Mountain Invasion Research Network (MIREN) (Bacaro et al., 2015; Haider et al., 2021). Thereby, three roads on the island's southern slopes were selected, spanning from the coast to the crater of Mt. Teide. By dividing the elevational range of each road into 19 equal portions, 20 sampling locations were chosen evenly over an ascent of approximately 2,350 m. At each sampling location, two plots with 2 m × 50 m each were established, one directly adjacent and with the long side parallel to the road (hereinafter roadside plot), and a second one perpendicular to the roadside plot, at 50–100 m away from the road (hereinafter distant plot). Steep topography and private property prevented sampling in some locations, resulting in a total of 111 plots.

Vegetation Relevées and Leaf Trait Sampling

In each plot, all vascular plant species were identified, and their cover was visually estimated and recorded via classes from 1 to 7 (where class 1 corresponds to a cover of up to 1%; 2 = 1–5%; 3 = 6–25%; 4 = 26–50%; 5 = 51–75%; 6 = 76–95% and 7 = 96–100%). For the leaf trait sampling, we primarily aimed to collect the most abundant and most frequent species, both native and non-native species, and all life forms (grasses, forbs, shrubs, and trees). For each species, we collected 10–50 leaves from one to three typical populations, so that the sampling covered the species' elevational distribution. Later, trait values of all populations of a species were averaged to get a single value for each trait of each species. With this approach, we obtained for each plot trait information of species making up at least 80% of the relative plot cover (except for five plots); an established threshold for a reliable characterization of the functional properties of a community (Pakeman and Quested, 2007). In total, 332 leaf samples for 189 species (out of 270 species recorded in the survey) were collected.

Leaf Trait Analysis

For a subset of the leaf samples, we conducted laboratory analyses of five traits associated with an acquisitive (SLA, leaf N, and leaf N:P ratio) and a conservative growth strategy (LDMC and leaf C:P ratio). In a second step, we used this subset of samples as "calibration samples" to predict the trait values for the remaining samples via the high-throughput method of near-infrared reflectance spectroscopy (NIRS) as described for example by Foley et al. (1998) and applied in previous projects (Kühn et al., 2021; see **Supplementary Material** for more details).

In the field, the calibration samples were kept in bags with a wet tissue to prevent water loss during the sampling day, then weighed and scanned with a flatbed scanner on the same day. Leaf area was calculated using WinFOLIA (Regent Instruments, Quebec, CA). The samples were subsequently dried for 72 h at 80°C in a drying oven and weighed again. LDMC was calculated as the ratio of leaf dry weight to leaf fresh weight, and SLA as the ratio of fresh leaf area to leaf dry weight (Kleyer et al., 2008; Pérez-Harguindeguy et al., 2013). The dry samples were milled into fine powder using a ball mill (MM 400, Retsch, Haan, DE). Following a nitric acid digestion and adding ammonium heptamolybdate and ascorbic acid, the concentration of leaf phosphorus was measured by visual assay with a Photometer SmartSpec$^{\mathrm{TM}}$ 3000 (BIO-RAD). The total carbon and nitrogen concentrations were measured via gas-chromatography with the Dumas method (Vario EL Cube, Elementar Analysensysteme, Langenselbold, DE). From these single element values, we calculated leaf N:P and leaf C:P ratio.

Species Richness, Community-Weighted Trait Means and Functional Diversity

Species richness was defined as the total number of species recorded per plot. Community-weighted trait means (CWMs) were calculated for each of the five leaf traits (SLA, LDMC, leaf N, leaf N:P ratio, leaf C:P ratio) and for each plot using the SYNCSA package (Debastiani and Pillar, 2012) in the R statistical environment (R Core Team, 2020):

$$\mathrm{CWM} = \sum_{i=1}^{N} p_i trait_i \tag{1}$$

where p_i is the relative cover of species i in the community, and $trait_i$ is the trait value of species i.

To quantify functional diversity (FD), we calculated Rao's quadratic entropy (Rao's Q; Rao, 1982; Botta-Dukát, 2005) for each plot:

$$\mathrm{Rao's\ Q} = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} p_i p_j d_{ij} \tag{2}$$

where the species' trait distances are weighted by their relative abundance in the community. N is the number of species in the plot, p_i and p_j are the relative cover of species i and j, and d_{ij} is the trait distance between species i and j. Thus, Rao's Q gives the mean functional distance between any two species in a plot. We included all five leaf traits in the calculation of Rao's Q (multi-trait functional diversity). The calculation of functional diversity was done with species trait values scaled to unit variance, in the FD package in R (Laliberté and Legendre, 2010). Elevational variation of CWMs, functional diversity (Rao's Q) and species richness and the effect of road proximity are shown in **Supplementary Figure 1** and **Supplementary Table 1**. See **Supplementary Table 2** for an overview of all the leaf trait, soil and temperature variables used in this study.

Soil Sampling and Soil Analysis

From three locations in each plot, we collected a bulked soil sample from the upper 10 cm of the mineral soil layer. The samples were sieved (<2 mm) and remaining, visible plant particles were removed. Soil pH was measured in 1 molar KCl. For gas-chromatographically measuring total soil carbon and nitrogen concentrations, fresh soil was dried for 72 h at 105°C and then milled to fine powder. To determine the concentrations of the base cations calcium, magnesium and potassium, fresh soil was percolated in a solution of 0.1 molar barium chloride. Afterward, the cation concentrations were measured by atomic absorption spectrometry (ContrAA 300 AAS, Analytik Jena, Jena, Germany). The concentration of hydrogen ions was measured by determining the pH of the percolates, and the cation exchange capacity (CEC) was calculated as the sum of ion equivalents of all measured exchangeable cations (Ca, K, Mg, and H). For the determination of bioavailable phosphorus, the soil samples were analyzed using the method proposed by Olsen (1954). Like for the leaf samples, the measurement of soil P was done photometrically after adding a molybdate reagent solution and an ascorbic acid solution. The ratios of N:P and of C:P were calculated.

Temperature Data

Near-surface soil temperature was measured using miniature loggers (HOBO 64K Pendant, with 0.53°C accuracy, Bourne, MA, United States). They were installed in the center of the roadside plot and 50 m away from it, at the start of the distant plot, at a depth of 3 cm below the soil surface. They were programmed to log at 1.5 h intervals from May 2018 until April 2019. Data quality control was done in R (R Core Team, 2020), and days with less than 13 measurements per day (out of 16) were excluded when calculating the daily averages, as well as months with less than 27 days with enough measurements. After data cleaning, daily and monthly averages and the bioclimatic variables Bio10 (mean temperature of the warmest quarter) and Bio11 (mean temperature of the coldest quarter) were calculated using the climvars package (Maclean and Gardner, 2021), which follows the definitions of WorldClim (Hijmans et al., 2005). By taking into account climate of different seasons and including the periods where temperature stress might be strongest for plant growth, survival and reproduction, these two bioclimatic variables are ecologically and physiologically relevant and are widely used in species distribution studies (Lembrechts et al., 2019). We did not choose to use annual means, for example, since they cover periods that have less influence on foliage. Temperature variation with elevation and proximity to roads is shown in **Supplementary Figure 2**.

Statistical Analysis

All statistical analyses were carried out in R version 4.1.0 (R Core Team, 2020). To get an overview about how the measured chemical soil properties are correlated, a principal component analysis (PCA) was performed (function "prcomp") and visualized with the R packages "FactoMineR" (Lê et al., 2008) and "factoextra" (Kassambara and Mundt, 2020).

To analyze elevational changes of chemical soil properties and the influence of road proximity, we used linear mixed-effects models (R package "lmerTest"; Kuznetsova et al., 2017). Models were fitted with each of the eight investigated soil variables as response, and elevation, proximity to roads (roadside or -distant plot) and their interaction as fixed effects. Sampling location nested in road identity was added as random factor. Elevation was scaled prior to analyses. Because the interaction term was never significant, a simplified model without the interaction was fitted. *P*-values were calculated from F-statistics of type III sum of squares with Satterthwaite approximation to estimate the denominator degrees of freedom. We visually checked, if model assumptions were met, and transformed the response variables if necessary. This was the case for soil N and soil Mg concentrations, soil C:P and N:P ratios (log-transformed) and for soil Ca and soil K concentration and CEC (square-root-transformed). Additionally, we fitted models with the same structure, but including the quadratic term of elevation, using its second-order polynomial. We compared the AIC (Akaike's Information Criterion) of linear and quadratic models and considered those with lower AIC as the better models. For soil pH, C:P ratio and K, the quadratic model had a lower AIC than the linear one.

To test and compare the effects of soil properties, temperature and road proximity, we used a multivariate generalized linear mixed model based on a Markov Chain Monte Carlo sampler ("MCMCglmm" function in the R package "MCMCglmm"; Hadfield, 2010). Response variables were the CWMs of SLA, LDMC, leaf N, leaf N:P, and leaf C:P, Rao's Q of these traits (multi-trait functional diversity) and species richness. All response variables were scaled and, if necessary, transformed prior to scaling. This was the case for SLA, leaf C:P ratio, Rao's Q, and species richness (log-transformed). As predictors we included temperature of the warmest and of the coldest quarter, proximity to roads (roadside or distant plot), and chemical soil properties, summarized by using the values of the first three axes of the PCA described above. We also permitted the interactions between road proximity and soil variables. All predictors were scaled, which allows to directly compare their effect sizes. The random effect of sampling location nested in road was added to control for the fact that all response variables were measured in the same plots. The model was fitted with a Gaussian distribution for all response variables. We ran the model with 100,000 iteration steps, discarded the first 10,000 samples as burn-in and thinned the remaining samples by storing every 25th iteration step.

RESULTS

Correlations Between Soil Properties

We found three major axes of variation in chemical soil properties. The first PCA axis summarized cation concentrations in the soil (hereafter referred to as "cation axis"), and explained 41.1% of the total variation (**Figures 2A,C**). The soil cations Ca, Mg and the CEC were strongly negatively correlated with the cation axis (i.e., high positive values along this axis mean a low concentration of soil Ca and soil Mg, and low CEC). The second

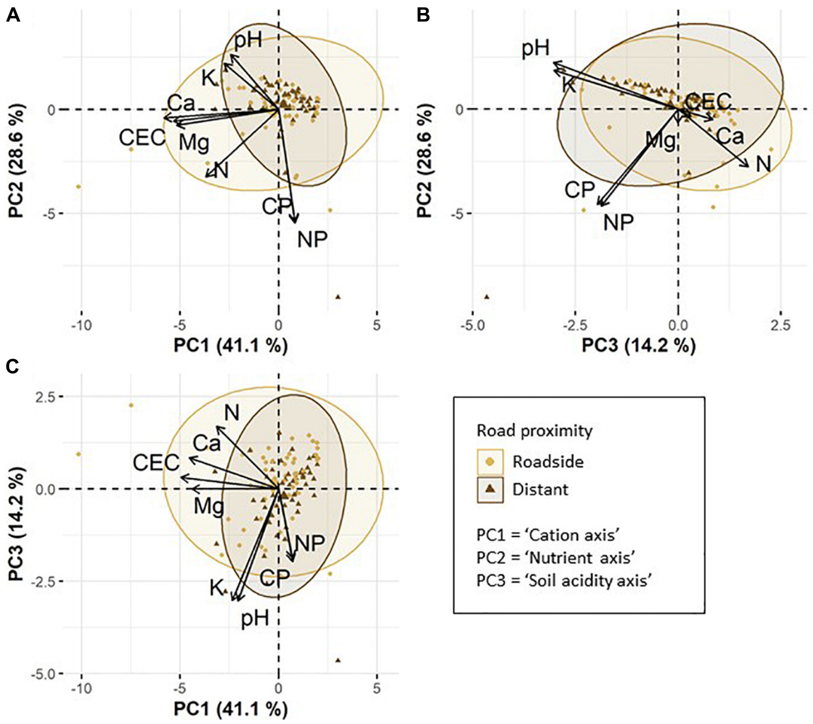

FIGURE 2 | Principle component analysis (PCA) based on eight chemical soil properties: pH, cation exchange capacity (CEC), soil nitrogen (N), carbon to phosphorus ratio (CP), nitrogen to phosphorus ratio (NP), calcium (Ca), magnesium (Mg), and potassium (K). Per cent values show the variance explained by PC1, PC2, and PC3. The ellipses are 95% confidence ellipses and visualize the variation in soil properties of roadside plots compared to distant plots. Larger symbols correspond to the ellipse centroids. **(A)** displays axes PC1 and PC2, **(B)** axes PC3 and PC 2 and **(C)** axes PC1 and PC3.

PCA axis summarized the concentration of soil N as well as soil C:P and N:P ratios (hereafter referred to as "nutrient axis"). This axis explained 28.6% of the total variation (**Figures 2A,B**). Soil N and both soil C:P and N:P ratios were negatively correlated with the nutrient axis. The third PCA axis summarized soil acidity and the concentration of soil K (hereafter referred to as "soil acidity axis"), explaining 14.2% of the total variation (**Figures 2B,C**). Soil pH and K were negatively correlated with the soil acidity axis.

Response of Soil Properties to Elevation and Road Proximity

Most of the investigated chemical soil properties (pH, Ca, K, Mg, and CEC) decreased significantly with increasing elevation (**Figures 3A,E–H** and **Table 1**). While soil Ca, Mg and CEC decreased continuously, pH and soil K decreased more steeply in the lower part of the elevational gradient. Soil C:P ratio peaked at mid elevations, while while soil N:P ratio did not respond to elevation (**Figures 3C,D** and **Table 1**). Soil pH values were significantly higher in roadside plots compared to distant plots (**Figure 3A** and **Table 1**). For concentrations of N and Mg, the opposite was observed (**Figures 3B,G** and **Table 1**).

Effects of Temperature, Soil Properties, and Road Proximity on Vegetation

Mean temperature of the warmest quarter had a positive effect on leaf N and a negative effect on leaf C:P ratio (**Figure 4** and **Supplementary Table 3**). Mean temperature of the coldest quarter had a positive effect on SLA and species richness (**Figure 4** and **Supplementary Table 3**). SLA increased with increasing CEC and soil cation concentrations (negative effect of the "cation axis," **Figure 4** and **Supplementary Table 3**). Species richness was higher in roadside plots compared to distant plots. Among the environmental variables tested, mean temperature of the coldest quarter had the strongest effects on vegetation characteristics (strongest on species richness, followed by the effect on SLA). The second strongest influence was exerted by mean temperature of the warmest quarter (on leaf N and second on leaf C:P ratio), followed by road proximity (on species richness) and soil cations (on SLA).

DISCUSSION

Elevational Responses of Chemical Soil Properties

Soil properties, such as soil acidity and the amount of nutrients, vary with environmental conditions and can reflect e.g., climatic differences, such as lower temperatures or aridity. This effect can be seen in particular along elevational gradients due to the strong environmental variation. In our study, the CEC, base cation concentrations (Ca, Mg, K) and pH decreased significantly along the elevational gradient on the south of Mt.

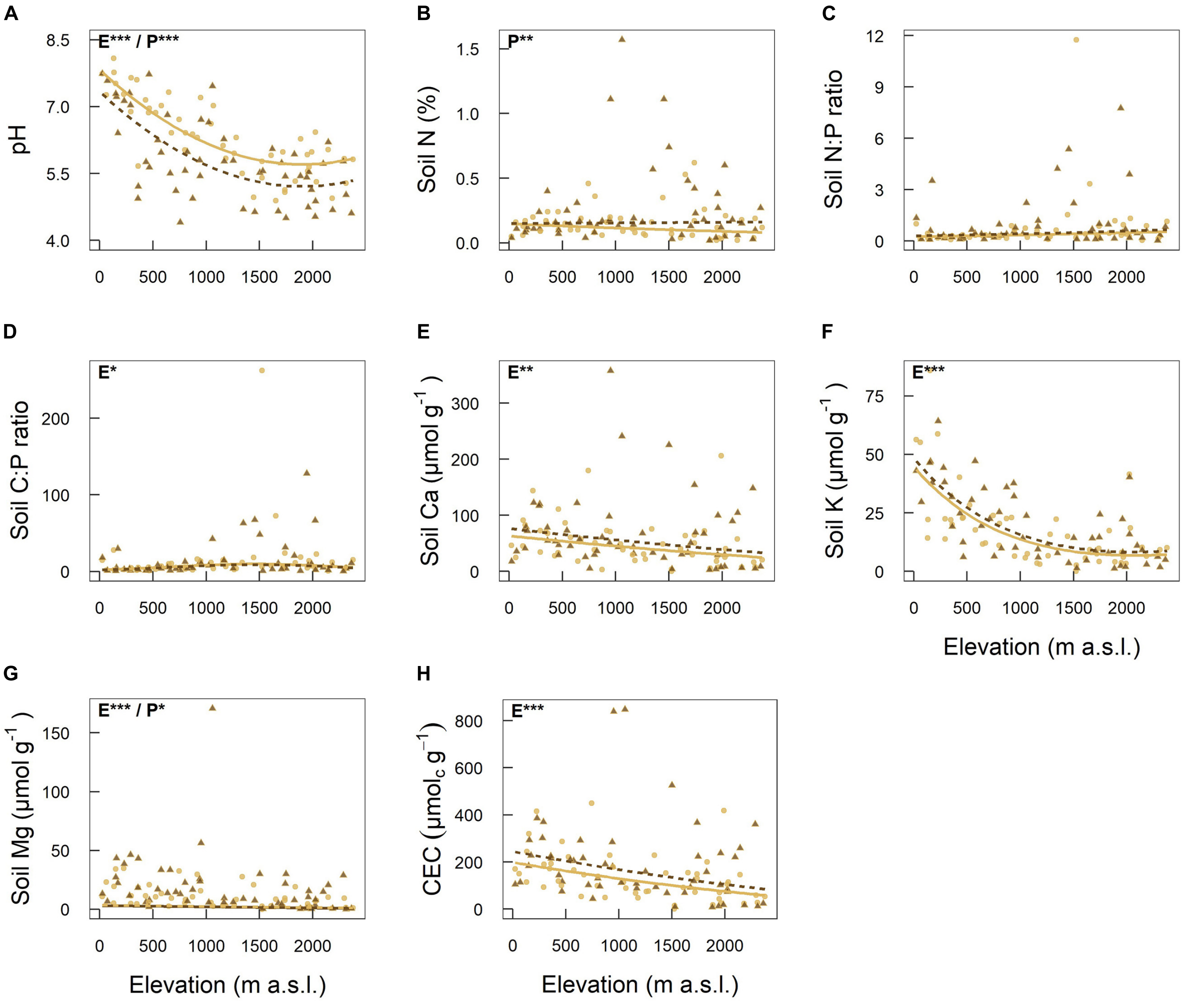

FIGURE 3 | Elevational variation of eight chemical soil properties: **(A)** pH, **(B)** Soil nitrogen, **(C)** Soil nitrogen to phosphorus ratio, **(D)** Soil carbon to phosphorus ratio, **(E)** Soil calcium, **(F)** Soil potassium, **(G)** Soil magnesium, **(H)** Cation exchange capacity. Linear mixed-effects models were fitted separately for each soil property as response to elevation (first or second-order polynomial), road proximity (roadside or distant plot), and their interaction. Sampling location nested in road identity, which corresponds to the sampling design, was included as random effect. Light brown continuous lines and circles represent plots next to the road (roadside plots). Dark brown dashed lines and triangles represent plots away from the road in natural vegetation (distant plots). For soil pH, C:P ratio and K the model including the quadratic term of elevation had a higher explanatory power (lower AIC) compared to the model including only the linear term of elevation. Significant terms are indicated at the top left of each plot: "E" corresponds to elevation and "P" to road proximity. Significance levels are indicated as follows: < 0.001 = ***, < 0.01 = **, < 0.05 = *.

Teide, on Tenerife. This elevational response of exchangeable cations is overall consistent with the results of another study conducted from mid to high elevations (1,400–3,100 m a.s.l.) on the northeastern slopes of Mt. Teide (Köhler et al., 2006), and was also found, for example, on the Galápagos Islands (Dinter et al., 2020). In contrast, a study conducted in the equatorial Andes did not observe clear elevational changes of soil base cations (Unger et al., 2012), and in Costa Rica, CEC, Ca and Mg concentrations were found to be highest at the highest elevation sampled (2,600 m a.s.l.; Grieve et al., 1990). Soil nutrient concentrations depend on multiple factors which do not necessarily change linearly with elevation (Körner, 2007). Differences in bedrock, soil age, climatic conditions or vegetation between regions result in region-specific soil property changes along elevational gradients (Köhler et al., 2006; Dinter et al., 2020). The distinct vegetational cover along the elevational gradient and thus input of litter between the study site in the equatorial Andes (Unger et al., 2012) and our study makes it difficult to sufficiently compare the results. Although soil age and bedrock are similar on the study site in Costa Rica, the investigated soil shows much greater depth and different structure. In contrast to our results, soil pH was found to increase from mid-elevations toward the tree line (around 2,100 m a.s.l.) on the northeastern slopes Mt. Teide (Köhler et al., 2006). These opposing responses of soil pH in the same mountain system are likely to be caused by different elevational patterns of precipitation: annual precipitation mainly increased within our studied elevational gradient from the coast (~100 mm/y)

TABLE 1 | Results from linear mixed-effects models to analyze the effects of elevation and road proximity (roadside or distant plot) on chemical soil properties.

		Estimate	DenDF	*F*	*p*
Soil pH	Elevation	0.253	48.882	50.797	**<0.001**
	Road proximity (distant)	−0.492	51.171	25.799	**<0.001**
Soil N	Elevation	−0.077	51.422	0.523	0.472
	Road proximity (distant)	0.333	51.017	7.392	**0.009**
Soil N:P ratio	Elevation	0.2303	50.206	3.266	0.077
	Road proximity (distant)	0.1777	50.658	1.393	0.243
Soil C:P ratio	Elevation	−0.323	50.240	4.895	**0.011**
	Road proximity (distant)	−0.1073	50.502	0.422	0.519
Soil Ca	Elevation	−0.896	51.058	7.912	**0.007**
	Road proximity (distant)	0.781	52.725	1.691	0.199
Soil K	Elevation	0.411	50.560	39.361	**<0.001**
	Road proximity (distant)	0.266	52.119	1.673	0.202
Soil Mg	Elevation	−0.721	50.436	27.108	**<0.001**
	Road proximity (distant)	0.350	51.889	4.224	**0.045**
CEC	Elevation	−1.937	51.334	15.963	**<0.001**
	Road proximity (distant)	1.560	52.923	3.021	0.088

Given are model estimates, denominator degrees of freedom (DenDF), F-values and p-values. To meet the model assumptions, soil N, N:P ratio, C:P ratio, and Mg were transformed using decadic logarithm, soil Ca, K, and CEC were transformed using the square root. For soil pH, C:P ratio and K the quadratic model (including the second-order polynomial of elevation), which had a lower AIC than the linear one, was used. Significant effects (p < 0.05) are highlighted in bold.

to the Canary pine forest belt (~500 mm/y), plateauing in the highest study plots. Vice versa, the lowest part of the elevational gradient studied by Köhler et al. (2006) was located in the most humid belt at mid-elevations (~1,050 mm/y), and precipitation decreased from there toward higher elevations (480 mm/y measured at 2,160 m a.s.l., in short distance to our highest study plots; Köhler et al., 2006). Hence, pH seems to be negatively correlated to precipitation and thus soil moisture—a pattern also reported from elevational gradients, for example, on Hawaii (Vitousek and Chadwick, 2013), on the Galápagos Islands (Dinter et al., 2020), and in Italy (Dixon et al., 2016).

In our study, soil C:P ratio peaked at mid-elevations, within the belt of Canary pine forest. A high ratio of soil C:P might indicate an efficient use of phosphorus by plants and microorganisms (Zhang et al., 2016), which in our case is likely to be supported by moister conditions in this elevational belt. On the other hand, the decomposition of pine needles is a slow process, potentially slowing down the relocation of phosphorus from dead plant material into the soil. At higher elevations, colder temperatures might further reduce decomposition rates and thus also increase the soil C:P ratio. The latter was found for example in a study in Northern China, where the soil C:P ratio significantly increased with elevation (Xu et al., 2019).

Soil N and N:P ratio showed no significant variation along our studied gradient. In our study, soil analyses were only conducted on mineral soil which has lower N concentrations than the organic layer. It was found that N concentrations were highest in the organic layers due to decomposing litter (Köhler et al., 2006). Thus, the increased litter in the pine forest resulted in higher N concentrations in mid-elevation soil samples which were, however, not significantly different to other samples. The lack of significant elevational changes of N and P coincides with the results of the studies conducted on the northeastern slope of Mt. Teide on Tenerife (Köhler et al., 2006), in the equatorial Andes (Unger et al., 2012), and on Mt. Gongga in China (Tan and Wang, 2016). Overall, young soils of volcanic origin—as found on Tenerife—are usually poorly developed and have lower nutrient concentrations and lower CEC compared to older soils (Köhler et al., 2006; Tan and Wang, 2016).

Road Effects on Soil Properties

We found significantly higher concentrations of soil N and Mg in plots away from the road compared to plots adjacent to the road. This contradicts our expectation that road verge soil

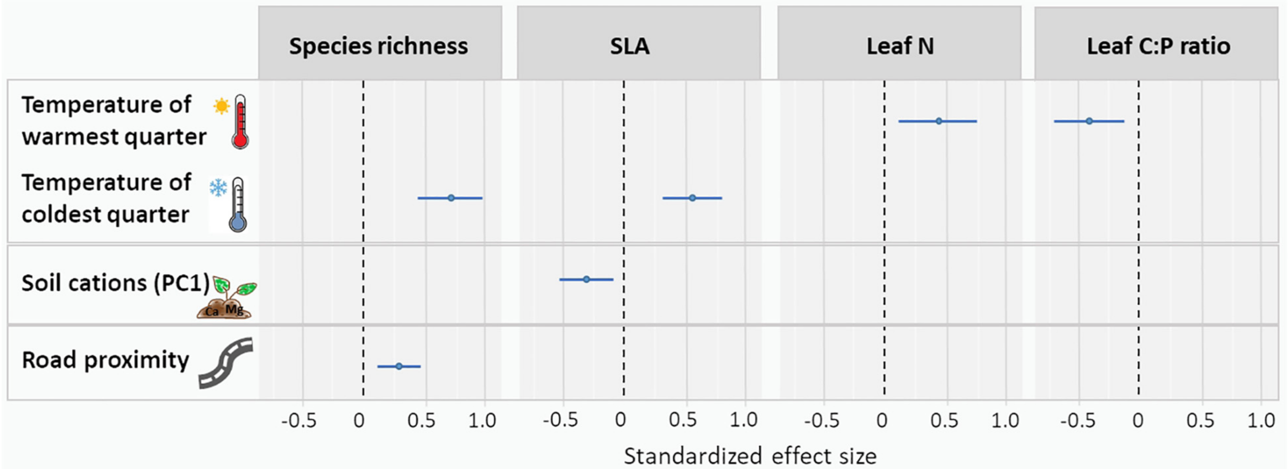

FIGURE 4 | Standardized effect size of mean temperature of warmest quarter, mean temperature of coldest quarter, soil cations (summarized through the first axis of the PCA) and road proximity on CWMs of plant functional traits (SLA [mm^2 mg^{-1}], leaf N [%], and leaf C:P [g g^{-1}]) and species richness. Only significant effects are shown. For complete results, see **Supplementary Table 3**.

might contain higher nutrient concentrations as a consequence, for example, of road dust, and moister conditions from road drainage (Forman and Alexander, 1998; Müllerová et al., 2011). Our results might be explained by higher bulk density caused by vehicles accessing the road verge which might lead to lower soil permeability and reduced water infiltration (i.e., greater surface runoff) and nutrient flows (Johnston and Johnston, 2004; Makineci et al., 2007; Deljouei et al., 2018; Yang et al., 2018). Other studies which investigated the effect of road proximity on soil nutrients also found higher soil N and soil Mg concentrations away from the road. They explained this increase of nutrients by higher vegetation density and higher N mineralization rates (Johnston and Johnston, 2004; Deljouei et al., 2018). Moreover, along roads, due to mechanical disturbance mainly through vehicles, finer soil material is removed and the more coarse-grained mineral soils have lower concentrations of the major nutrients P and N as well as of exchangeable cations (Johnston and Johnston, 2004).

Besides the resulting disturbance along roadsides, the construction of roads might have severe impacts on soil properties. These are especially strong, if alkaline construction material is used in naturally acidic environments (Müllerová et al., 2011). As a consequence, soil pH might increase next to roads—as found in our study as well as in other regions (Müllerová et al., 2011; Deljouei et al., 2018).

Effects of Temperature, Soil Properties, and Road Disturbance on Vegetation

The main objective of our study was to evaluate how two important components of the environment—chemical soil properties and temperature—shape vegetation characteristics along the elevational gradient on Tenerife. Moreover, we analyzed the influence of roads on vegetation, and how the effects of soil are modified through road proximity. The strongest effects detected on different vegetation aspects analyzed in this study was that of temperature of the coldest and the warmest quarter, both variables decreasing with elevation. Species richness and SLA (acquisitive trait) significantly increased with mean winter temperature, and leaf N (acquisitive trait) increased with mean summer temperature, while leaf C:P ratio (conservative trait) decreased with mean summer temperature (**Figure 4**). The effect of temperature on vegetation has been widely studied (Kreft and Jetz, 2007; Šímová et al., 2011) and shifts of functional traits toward more conservative trait values with decreasing temperature and increasing elevation have been observed (Read et al., 2014; Schellenberger Costa et al., 2017). Cold temperatures and frost affect plants by causing physiological stress via cellular membrane damage on leaves and lower metabolic activities, which ultimately affect growth and reproduction (Yadav, 2010). At the high elevations of Mt. Teide, where we measured winter temperatures in the soil as cold as 1.7°C (**Supplementary Figure 1**), the specialist sclerophyllous shrubs dominate the communities. They seem to cope with the extreme conditions by having smaller, thicker leaves (low SLA) with high carbon content and low nutrient concentrations (high leaf C:P ratio). Because only specialist species are able to survive in such harsh conditions at high elevations, temperature also has a filtering effect on species richness toward colder sites, as observed as well on the neighboring island La Palma (Irl et al., 2015), but also at the global scale (Wright et al., 2017; Bruelheide et al., 2018) and e.g., for Kilimanjaro (Peters et al., 2019).

In our study, soil cation concentrations were the only chemical soil properties exerting a significant effect on the vegetation. SLA increased with increasing soil Ca and Mg concentrations and higher CEC. More favorable soil conditions at low elevations (with higher Ca, Mg and CEC) seem to be an important factor—in addition to temperature—which favors species with acquisitive strategies in these communities. As soil conditions get less favorable (i.e., with increasing elevation), the communities are characterized by a more conservative strategy with smaller SLA. Our results deviate from those found at Mt. Kilimanjaro, where soil nutrients (N, P, Ca, Na, K) had a negative effect on LDMC, and a positive effect on leaf N and leaf P, but not on SLA (Schellenberger Costa et al., 2017). Despite the different single results in terms of which traits were affected by soil nutrients, the overall result from Schellenberger Costa et al. (2017) also showed soil nutrients to favor acquisitive strategies. The lack of an effect from our soil nutrient axis (determined by soil N concentration, soil C:P, and N:P ratio) might be due to the very low concentrations of N and P in the poorly developed soils at Mt. Teide, as also found in another study on Tenerife (Köhler et al., 2006) and in general for Mediterranean soils (Torrent, 2005). Even though the N concentrations we found in the soil along the southern slopes of Tenerife spanned over a wide range (0.02–1.57%), comparable to values found in alpine grasslands (0.2–1.2%, Körner, 2003), only a few sites contained more soil N than found by Köhler et al. (2006) on the northern slopes (0.02–0.26%). The overall poorly developed soils in our study area might also explain why we could not detect a positive effect of soil quality on species richness, although soil quality, and species richness almost paralleled each other along the elevational gradient.

We expected species richness to be higher next to roads compared to non-road habitats, because of the disturbance in the roadside habitats, which should weaken competition among plants through an increase in bare soil, light and nutrients and thus allow more species to co-exist (Catford et al., 2012). Indeed, communities at the road verge harbored more species than the communities away from the road in our study region. However, this was likely primarily because of the heterogeneity generated by the human activities associated to roads and more available space and light than increased nutrient availability (lower soil N and Mg concentrations next to the road). The removal of biomass during road maintenance in Tenerife, for example, prevents continuous cover of highly competitive species by creating empty spots and opportunities for other species to grow. We also expected disturbance to favor species with acquisitive traits, such as high SLA (McIntyre et al., 1999; Bernhardt-Römermann et al., 2011), which allow for a quick uptake and use of the available resources. However, in our study, CWMs of the functional traits investigated did not differ between communities next to and away from the road. This finding, together with the absence of an increase in functional diversity next to roads, is surprising and difficult to explain. A possible explanation might be that during the warmest quarter, temperature in roadside plots was significantly higher than in distant plots (**Supplementary Material**). Therefore, the effect of road disturbance might have been captured by the overall stronger effect of temperature. Alternatively, the new species favored by disturbance at roadsides might be functionally similar and thus do not significantly contribute to functional diversity or changes in CWMs.

We found no support for the expected interaction between soil properties and road disturbance. We expected that the same amount of soil nutrients or cations, for example, would have a different impact on roadside vegetation compared to the distant vegetation, as these nutrients might not be available for plant uptake due to higher soil compaction from vehicle traffic. However, these interacting effects were not detected in any of the vegetation characteristics analyzed here. This might be explained by a less pronounced difference in soil bulk density or grain size distribution in roadside and distant plots than we presumed, and therefore a similar nutrient availability for plants.

Surprisingly, functional diversity was not affected by any of the environmental factors considered. While other studies found functional diversity to decrease through environmental filtering (but see Schellenberger Costa et al., 2017; López-Angulo et al., 2020; Midolo et al., 2021) and to increase through competition (Mayfield et al., 2010), we could not detect any differences. While our selection of traits was done carefully in order to encompass the aspects of vegetation relevant to our questions, we also acknowledge that the leaf traits considered in this study do not capture all dimensions of trait variation. For example, seed and dispersal traits may also be important for community assembly (although not independent of life form, which make them less suitable for this study). Since the studied environmental variables are all related to competition intensity, we consider the choice of leaf traits related to the species' competitive and growth ability as appropriate. Thus, regarding the lack of a response of functional diversity in the study presented here, we emphasize that although elevation is often criticized of being merely an umbrella term, it also combines the effects of several environmental factors, thus revealing ecological patterns which cannot be detected with individual analyses of single variables (Körner, 2007).

In our study, temperature had the strongest effects on vegetation characteristics along the elevational gradient of Tenerife—stronger than chemical soil properties and disturbance. As decreasing temperature gradients are general patterns in mountains, our findings align with observations from elevational gradients in other regions. In addition, our results are in accordance with studies explaining broad-scale patterns of species richness and functional trait variation, particularly those looking at continental and global geographic patterns (Hawkins et al., 2003; Kreft and Jetz, 2007; Šímová et al., 2011; Bruelheide et al., 2018). While elevational temperature patterns are quite linear, soil patterns are more idiosyncratic, and thus not simply transferrable across regions. At the species level, a global compilation study found soil measured at the same site as the traits to be a more important driver of trait variation than temperature data extracted from smaller resolution sources (Ordoñez et al., 2009; but see Liu et al., 2017). Together with the results from our regional study, this emphasizes the importance of including data from local environmental conditions for climate, disturbance and fine-scale soil conditions for understanding biodiversity patterns.

DATA AVAILABILITY STATEMENT

The dataset presented in this article is available at the Dryad Digital Repository, https://doi.org/10.5061/dryad.66t1g1k37.

AUTHOR CONTRIBUTIONS

AR, LF, and SH conceived the ideas and analyzed the data. AR conducted the fieldwork and collected the vegetation data with help from SH and JA. AR and LF conducted the chemical soil and leaf analysis, respectively. AR and LF led the writing with important contributions from SH. All authors revised drafts of the manuscript, and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.758160/full#supplementary-material

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Beyond the Tree-Line: The C_3-C_4 "Grass-Line" Can Track Global Change in the World's Grassy Mountain Systems

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von Humboldt's tree-line concept has dominated mountain ecology for almost two hundred years, and is considered a key indicator for monitoring change in biome boundaries and biodiversity shifts under climate change. Even though the concept of life zones and elevation gradients are a globally observed phenomenon, they have not been thoroughly explored for many contexts. One such example is the tree-line ecotone, a widely used conceptual tool to track climate change in many regions, which has limited application in the widespread tree-sparse, grassy systems that comprise a third of the world's mountain systems. Among grasses (Poaceae), temperature is linked to variation in photosynthetic performance and community dominance for C_3 and C_4 metabolic groups, due to its role in limiting photorespiration in the C_3 photosynthesis process. Here, we investigate this community shift in grassland-dominated mountains to demonstrate the role of climate in driving this transition and discuss the potential applications of this tool to mountain ecosystem conservation worldwide. For identifying grass-dominated mountains worldwide, we measured the grass-cover using satellite data. We then compiled Poaceae distribution data for ten grass-dominated mountains spanning from 42°S to 41°N and determined the temperature intervals and elevation ranges at which each genus was found, testing for effects of temperature, precipitation, and latitudinal gradients on the dominance of C_3-C_4 grasses. Temperature was the main driver of C_3 dominance, with the richness of C_3 genera tending to surpass the taxonomic dominance of C_4 plants along mountain temperature gradients where the annual mean temperature was colder than ca. 14.6°C. Similar patterns were observed in eight out of ten mountains, suggesting that this may constitute an isotherm-driven ecotone. Consequently, this C_3-C_4 transition offers a promising tool for monitoring climate change impacts in grassy mountains. C_3-C_4 grass community shifts in response to environmental change will likely have major implications for fire frequency and severity, rangeland productivity and livelihoods, food security, and water budgets in mountain systems. Given the severity of the implications of global change on these social-ecological systems, we propose that a "grass-line" monitoring protocol be developed for global application.

Keywords: taxonomic diversity, photosynthesis, mountain ecology, environmental gradients, climate change, community shift, Poaceae (=Gramineae)

INTRODUCTION

Mountains and Arctic systems are experiencing climate change faster than any other natural systems (Dirnböck et al., 2011; Steinbauer et al., 2018; Hock et al., 2019). In mountain systems characterized by woody natural vegetation, the tree-line is one of the primary indicators that ecologists use to measure the ecosystem-level impacts of rising temperatures, with a similar approach being followed at high boreal latitudes viz. the forest-tundra timberline (Körner, 2012; Paulsen and Körner, 2014). One of the most valuable applications of the tree-line as a tool to track climate change is that—whenever the tree-line is physically present (whether as a broad ecotone or a sharper transitional zone)—it provides a clear visual reference which can be globally assessed (Körner, 2012) and tracked over time (Paulsen and Körner, 2014). Even though this tree-line-based isotherm tracking has considerable merit in regions where there is a physical tree-line, it has limited practical applicability in regions that are naturally tree-sparse and dominated by grasslands, and which thus demand more locally suited methods and approaches (Veldman et al., 2015; Bond, 2016) in order to be equally understood under scenarios of past and future climatic change.

Tree-line studies have historically been centered in temperate regions of the northern hemisphere, which—for the most part—has woody vegetation (Woodward et al., 2004). The tropical areas of the Americas, Africa, and Asia also have a clear transition between woody and non-woody vegetation with elevation (Woodward et al., 2004; Körner, 2012). However, many sub-tropical and temperate systems in the southern hemisphere do not show the same pattern and are dominated by natural grassy systems, in contrast to their northern temperate and tropical equivalents (Woodward et al., 2004; Edwards and Still, 2008; Sylvester et al., 2017; Carbutt, 2019). As a result, the tree-line concept has limited to no practical value in monitoring global change in these systems.

After forests (34%), grasslands (18%) are the dominant land-cover in the World's mountains, with almost a third (31.6%) of the World's mountains having a larger proportion of grassland cover than forest cover (**Figure 1**). Grassiness is therefore an ecologically fundamental component of mountain systems globally, with grass-dominated (e.g., grasslands, meadows) or co-dominated biomes (e.g., savannas, steppes) occurring in mountain regions of all continents (except Antarctica; **Figure 1**). Although the World's most grassy mountains occur in Central Asia, southern Africa, southern South America, and New Zealand, almost all mountains worldwide have some element of grassiness (**Figure 1**). Grassy mountains provide valuable ecosystem services (Parr et al., 2014); and entire cultures, livelihoods, and various forms of indigenous food security strategies have evolved in and around such grassy mountain systems (Maurer et al., 2006; Lavorel et al., 2011). Such montane grasslands are also often paleo-grasslands, with high floristic endemism and richness (Van Wyk and Smith, 2001; Mucina and Rutherford, 2006; Clark et al., 2011; Parr et al., 2014), while natural montane forest in these grassy mountains may be confined to lowest elevations in topographically suitable locations and fire refugia (Mucina and Rutherford, 2006; Adie et al., 2017).

In the absence of a woody component, developing alternative tools to track how communities in grassy mountains are being transformed by anthropogenic climatic change is necessary to ensure specific grassland-focused conservation strategies. Changes in physiological characteristics and/or the functional composition of grass communities can provide valuable insights on how changes in environmental gradients (like temperature) affect grass species ranges in mountains. One such relationship is the well-known differential niche occupancy of C_3 and C_4 grasses along temperature gradients. Indeed, empirical data shows that the relative taxonomic richness of C_3 grasses gradually increases with decreasing temperature found along elevation gradients (Tieszen et al., 1979; Bremond et al., 2012). This phenomenon is a result of a narrow niche overlap of the temperature tolerances in the abiotic component of the realized niches between these two photosynthetic groups, mainly due to the differences in temperature response of instantaneous net CO_2 assimilation rate and the regeneration potential of ribulose bisphosphate (RuBP) and ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) between these groups (Sage and Kubien, 2007). Available data suggests that, under enriched CO_2 environments, the Rubisco regeneration limits the distribution of C_3 plants, while CO_2 assimilation rates is restrained by temperatures lower than 20°C in C_4. While this limitation seems to be a key driver of C_3 habitat suitability, occasional acclimation to cooler environments in C_4 is empirically observed, through positive regulation of Rubisco and C_4 cycle enzymes (Sage and Kubien, 2007). However, due to its close relation to a specific temperature threshold in C_3, it is possible to identify the temperatures where most C_3 grasses have their lower elevational limits of their distributions, and to track changes in the community transition to C_4 along temperature gradients. This concept, proposed here as the "grass-line," can be tested and implemented in grassy mountain regions and provides a valuable tool to monitor temperature changes in grass dominated mountain regions where this transition occurs.

The aim of this study was, therefore, to test for a consistent/general relationship between environmental conditions and the elevation where colder temperatures allow C_3 grasses to dominate over C_4 genera. Specifically, we aim to test

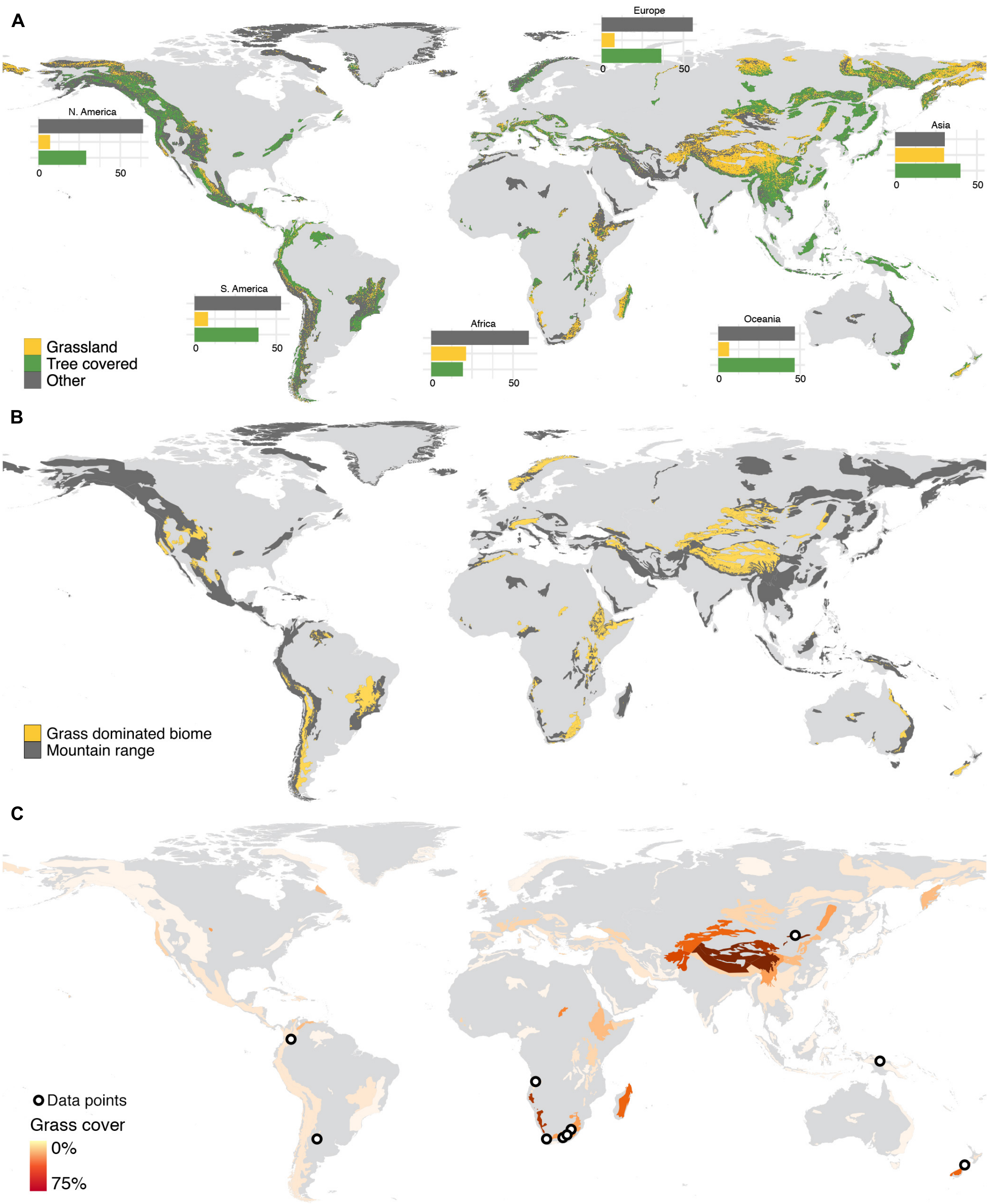

FIGURE 1 | Grassiness in the World's mountains. **(A)** Proportion of area occupied by grasslands, forests, or other land cover categories in each mountain. **(B)** The spatial distribution of grass-dominated and co-dominated biomes in the World's mountains. **(C)** Percentage of land in each mountain covered by grassland and data localities used in the elevation vs. C_3 -C_4 community change analysis.

if the temperature where Poaceae C_3 genera richness becomes higher than C_4 genera richness along elevation gradients is driven by similar temperature thresholds across grassland-dominated mountains, and thus, linked to an isotherm and to demonstrate how it can be applied to track temperature change in mountains missing a tree-line. By gathering distribution data for C_3 and C_4 genera of grasses on different grass-dominated or co-dominated mountains worldwide, we demonstrate the potential of using the isotherm thresholds of this ecological shift in different grassy mountains to keep track of isotherms over time and provide a basic framework to the development of a specific tracking protocol. Grassland-dominated mountains from southern

Africa, Asia, Australia, and the Americas consistently show a transition from C_4 to C_3 plants along elevation. This relationship between decreasing temperature and the C_3-C_4 ratio along elevation indicates an unambiguous link between community photosynthetic strategy turnover and the elevational distribution of isotherms. For grassy mountains this is as ecologically significant as the tree-line, and consequently we propose the C_3-C_4 dominance turnover transition as a "grass-line": a consistent ecological phenomenon conceptually comparable to the tree-line in that it can easily be used to monitor the ecological impacts of short- or long-term climate change using, for example, satellite imagery (Shoko et al., 2016), carbon isotopes (Collatz et al., 1998), and pollen record (Ehleringer et al., 1997; Collatz et al., 1998).

MATERIALS AND METHODS

Grass Dominated Mountains Identification and Site Selection

To identify which mountains are grassland-dominated and have low tree cover, we combined two strategies. First, we used the Global Mountain Delimitation shapefile (Rahbek et al., 2019b) and the Global Land Cover-SHARE (GLC-SHARE) databases developed by the Food and Agriculture Organization of the United Nations (FAO) (Latham et al., 2014). We summarized the land cover classification for each mountain, and then calculated the ratio of grasslands and forests cover using QGIS version 2.8.4 and R package "raster" version 3.5-2 (Hijmans, 2021). We then identified which grassland biomes were located within mountain ranges by overlaying the global mountain delimitation shapefile with the WWF's World Grassland Types (Dixon et al., 2014). We followed Dixon et al. (2014) definition of grassland biomes: "a non-wetland type with at least 10% vegetation cover, dominated or co-dominated by graminoid and forb growth forms, and where the trees form a single-layer canopy with either less than 10% cover and 5 m height (temperate) or less than 40% cover and 8 m height (tropical)."

Biodiversity Dataset Compilation and Preparing

For sampling the occurrence data, we used both published literature surveys and the Global Biodiversity Information Facility (GBIF), as listed in **Table 1**. We selected localities comprising 40°S–41°N to represent the latitudinal range of sub-tropical to tropical mountain grasslands: the Andes (Mount Córdoba and Transecto Sumapaz, in Cordillera Oriental), the Mongolian Mountains in China, the New Guinea mountains, and southern Africa's Eastern Great Escarpment (Cape Midlands Escarpment, Mpumalanga Limpopo, Maloti-Drakensberg, Manica Highlands) and Angola's Western Escarpment. We extracted individual geographic polygons for each mountain and used the contour of the elevation features to isolate individual mountains that were generalized within larger mountain groups in Rahbek et al. (2019b). The resulting spatial polygons used for the extant analysis are available as **Supplementary Material 1**. For GBIF data, we configured the occurrence record search to include all Poaceae within each of the grassland-dominated mountains identified in the previous step, and restricted search parameters to include only preserved specimen records with geographic coordinates. We checked spelling and authorship for all genera using the collaborative database Taxonomic Name Resolution Service v4.0[1], provided by IPlant. Once we obtained a set of records for each mountain, we removed putative geographic outliers and imprecise coordinates using the R package "CoordinateCleaner" (Zizka et al., 2019). Since the intervals of occurrence were calculated without taking into account the year of sampling, it is possible that the distributions

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[1] https://tnrs.biendata.org/

TABLE 1 | Mountain grasslands and the countries in which they occur, coordinates, number of C_3 and C_4 genera, climatic type (Köppen-Geiger), and the literature source for the species lists and occurrences.

Locality	Mountain	Coordinates	C_3 genera	C_4 genera	Climatic type (Köppen-Geiger)	Source
Angola	Angolan Western Escarpment	11°30′S, 15°00′E	2	43	Sub-tropical highland variety (Cwb)	GBIF (10.15468/dl.rvu7vl)
Argentina	Mount Córdoba	32°30′S, 65°00′W	18	33	Humid sub-tropical (Cwa)	Cabido et al. (1997)
China	Mongolian Mountains	41°00′N, 110°00′E	18	11	Cold semi-arid (Bsk)	GBIF (10.15468/dl.l4sr6i)
Colombia	Cordillera Oriental	04°00′N, 74°30′W	52	41	Tropical wet and dry (As)	Giraldo-Canas (2010)
Lesotho, South Africa	Maloti-Drakensberg	29°00′S, 28°00′E	32	49	Sub-tropical highland variety (Cwb)	GBIF (10.15468/dl.nz4g3n)
Mozambique, Zimbabwe	Manica Highlands	32°30′S, 19°00′E	22	62	Humid sub-tropical (Cwa)	*Flora Zambeziaca*, Harare Herbarium (SRGH)
New Guinea	New Guinea Mountains	04°00′S, 141°00′E	55	62	Temperate oceanic (Cfb)	GBIF (10.15468/dl.l2hfex)
New Zealand	New Zealand Mountains	42°00′S, 172°00′E	45	14	Temperate oceanic (Cfb)	GBIF (10.15468/dl.hfvhiz)
South Africa	Cape Midlands Escarpment	32°00′S, 25°00′E	27	33	Cold semi-arid (Bsk)	Schonland Herbarium (GRA)
South Africa, Mozambique, Eswatini	Mpumalanga Limpopo	31°00′S, 26°30′E	19	66	Sub-tropical highland variety (Cwb)	PRECIS, Buffelskloof Nature Reserve Herbarium (BNRH)

of some genera were overestimated. Similar studies in regions with more abundant and refined records should ideally account for the year of the collection, since the temperature increased considerably in some regions during the past decades. The complete record dataset used is available in **Supplementary Material 2**. We determined the photosynthetic pathway for each genus using literature sources (Osborne et al., 2014; Fish et al., 2015), and from this calculated the proportional occurrence of C_3 and C_4 genera for each elevational interval for each mountain (**Supplementary Material 3**).

Grass Dominated Mountains in Africa

Of the 18 major mountain regions delimited for Africa by Rahbek et al. (2019b), nine have greater areas of grasslands than forests: (1) Atlas Mountains, (2) Dafur Highlands, (3) East African Highlands, (4) Ethiopian Highlands, (5) Jos Plateau, (6) Namibian/Angolan Escarpments, (7) North Somali Mountains, (8) Southern Cape region, and (9) Madagascar Highlands. African grassy mountains extend from 35°S to 35°N, and are found within the steppe, savanna, grassland, bushveld, forest, and woodland biomes.

Grass Dominated Mountains in South America

In South America, grass-dominated mountain regions (i.e., mountains with greater areas covered by grasses than forests) were found in Patagonia, Central Western Andes and the Brazilian Central Plateau. We identified eight mountains harboring grassy biomes (**Figure 1**): (1) Brazilian Central Plateau (Cerrado) and (2) Atlantic Mountains (Campos Rupestres); (3) Bolivian Huanchaca Plateau, (4) Southern Andes and Western Central Andes (Punas and Andean steppe), (5) Northern Andes (Páramos), (6) Peruvian-Bolivian Yungas (Puna, Espinal and Andean steppe), (7) Patagonia (Patagonian steppe) and (8) Venezuelan-Brazilian Tepuis (Llanos, Campinarama, Guianan savanna, and Pantepui). In these mountains, the dominant vegetation types included steppes, grasslands, shrublands, and savannas. The Cerrado, for example, has a savanna-like vegetation, harboring grasslands, shrublands and forests. There are 470 native species of Poaceae listed for the Cerrado (Filgueiras, 2021), and the most common C_4 genera are *Paspalum*, *Panicum* (C_4 and C_3), *Axonopus*, *Mesosetum*, *Aristida* (C_4 and C_3), *Andropogon*, *Eragrostis*, *Digitaria*, and *Sporobolus*. Native C_3 genera, like *Ichnanthus*, *Echinolaena*, are rare (Filgueiras, 2021).

Grass Dominated Mountains in Asia

In Asia, grass-covered mountains are found in the west (the Sinai, the Iranian Mountains, the South Western Arabian Mountains, and the Ural Mountains); in Central Asia (the Tibetan Mountains, the Pamir-Tian-Shan Highlands, the Hindu-Kush Highlands); in the North (Siberian Mountains and the Kamchatka), and in Eastern continental Asia (Qilian Mountains, Shanxi Mountains, and the Inner Mongolian Mountains), predominantly in the form of steppes, but also as bushlands and meadows. In insular Asia, grasslands are found in the alpine regions of Borneo and New Guinea, but forest cover is dominant in both mountains. In continental Asia, due to the temperate environmental conditions found in most mountains, C_3 grasses tend to dominate.

Grass Dominated Mountains in Australia/New Zealand

In Australia and New Zealand, grassland-dominated mountains are concentrated in New Zealand and in the central-western mountain regions of Australia: Flinders Range, Macdonnell Ranges, and Pilbara-Hamersley Ranges. In the Flinders Range, the southern parts of the mountains are occupied by Mount Lofty woodlands, and the western portions of Macdonnell Ranges are occupied by the Great Sandy-Tanami desert, both including grasslands as vegetation types. For Australia, the GBIF database most-recorded Poaceae genera include three C_3 genera, namely *Austrostipa*, *Rytidosperma*, and *Poa*; one C_4 genera (*Eragrostis*); and one genera with both mechanisms, *Aristida*. Meanwhile, in New Zealand, the five most sampled Poaceae genera are all C_3: *Poa*, *Rytidosperma*, *Chionochloa*, *Agrostis*, and *Festuca*.

Spatial Analysis and Statistical Analyses

We extracted the elevation data for each record using the ASTER Digital Elevation Model (30 m resolution) and then identified the elevation range (minimum and maximum values) occupied for each genus in each mountain to create presence-absence matrices using intervals of 50 meters of elevation, assuming that each genus has the potential to occupy all the elevation bands within this interval (**Supplementary Material 4**). We used WorldClim (Fick and Hijmans, 2017) bioclimatic variables (annual mean temperature, maximum temperature on the warmest month, minimum temperature in the coldest month, annual precipitation, minimum precipitation on the driest month, maximum precipitation on the wettest month) to calculate the environmental space for each elevation interval of each mountain. For this, we converted the raster layers into spatial points for each set of mountains and then reclassified them according to the same elevation intervals used in the previous step, and then calculated mean values for each variable. The WorldClim 2.0 bioclimatic variables are based on multiple weather stations' data from over 25 years between 1970 and 2000, interpolated and spatially adjusted with geographic covariates. Annual mean temperature (bio 1) is calculated as the annual mean temperature for each station during the timeframe and their mean value over the years. Maximum and minimum temperature on the warmest and coldest month (bio 5 and 6, respectively) applies the same mean temperatures dataset to identify annual extremes and average them over this timeframe. It is important to note, however, that annual mean temperature might be a biased feature at high elevations because of the great diurnal and seasonal temperature variation in mountains. Thus, this predictor requires careful interpretation, with maximum annual temperature being more biologically meaningful when discussing climatic pressures on plants' survival and development because it is a more direct proxy to the growing season, along with precipitation. The information about elevation, temperature, precipitation, and the proportion of C_3 in each elevation

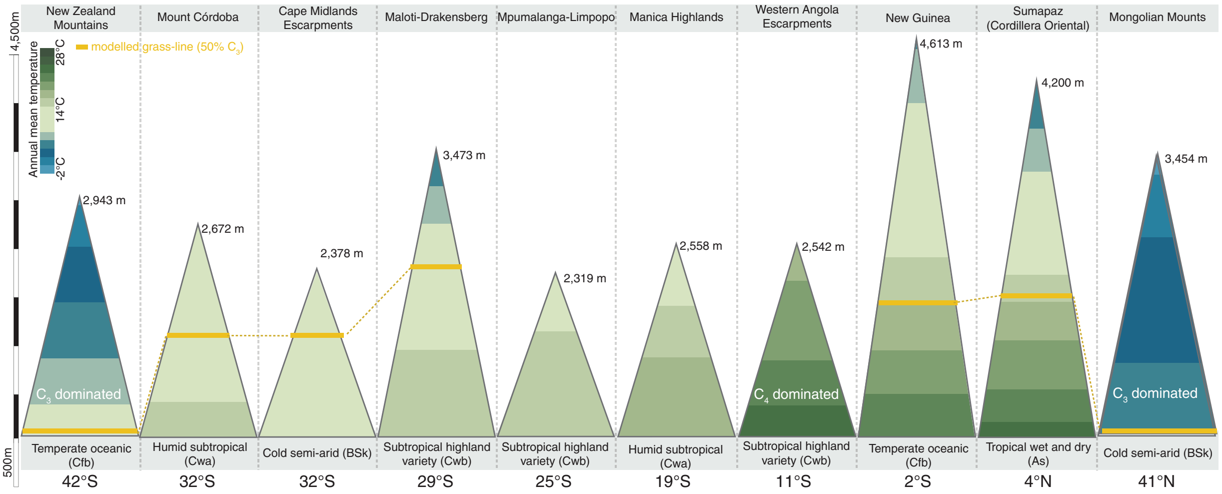

FIGURE 2 | Proportional C_3 composition of grasslands communities along elevation gradients in grassland dominated mountains in Asia, New Zealand, Africa, and South America, and their respective elevation, temperature, climates, latitudes, and modeled grass-line (50% of the genera on the community presenting C_3 photosynthesis).

range was then summarized in a single dataset, which we used to generate the plots and to produce generalized linear mixed models (GLMMs).

At the global scale, data were analyzed using GLMMs implemented using the glm() function, available in the base package "stats" (version 3.6.2) in the base R (R Core Team, 2020). We modeled the proportion of C_3 genera in relation to various predictors in models that incorporated a binomial distribution (with events/trials structure) and logit link function. Both slopes and intercepts were estimated for the relations between the proportion of C_3 genera and various continuous environmental variables in each mountain region. In order to minimize the effect of outlier sites where regional factors could create noise on the performance of GLMMs, mountains with *p*-values for individual models higher than 0.05 were removed from the global analysis steps. We eliminated variables that showed excessive collinearity (VIF > 5) and then used AIC criteria to select among plausible competing models. The sensitivity and specificity of each predicted threshold for annual mean temperature, maximum temperature in the warmest month and annual precipitation were calculated using confusion matrices created with the grass-line (i.e., the proportion of C_3 grasses passes 50%) indicated by the GLMMs and the number of true/false positives/negatives (i.e., $C_3 > 0.5$ sites) and the empirical dataset. All model fitting and sensitivity calculations are available as the **Supplementary Material 6**.

RESULTS

All continents except Antarctica host mountain-associated grass cover or grass-dominated biomes, but only in African mountains does grass cover occupy a larger area than forests (**Figures 1A,B**). Proportionally, individual mountain regions dominated by grasslands (>50% of the area covered by grasses) were concentrated in Southern Africa, Central Asia, and New Zealand (**Figure 1C**).

We identified a consistent relationship between the temperatures found at the elevation intervals at which C_3 grasses become more representative than C_4 genera. By gathering distribution data for 245 genera of grasses (122 C_3 and 123 C_4) on 10 different grass-dominated or co-dominated mountains (**Table 1**), spanning 42°S to 41°N, we show how the temperature thresholds of this ecological shift are related to steppe-like climatic conditions found along elevational and latitudinal gradients. There is a clear latitudinal gradient in the C_3-C_4 threshold elevation (i.e., the elevation at which dominance switches from C_3 to C_4) (**Figure 2** and **Supplementary Material 7**). As expected, C_3 genera were typically associated with colder temperature conditions, becoming more dominant as temperatures decrease along the elevation gradient (**Figure 3**). Since there was a weak effect of annual mean temperature ($p > 0.05$) on C_3 proportion in Manica Highlands and Western Angola Escarpments, it was not possible to fit grass-line values for these mountains (**Table 2**). Single-factor models for annual mean temperature, the maximum temperature in the warmest month, and annual mean precipitation returned *p*-values lower than 0.05 (**Table 3**). Individual mountains models had grass-line temperature thresholds ranging from 8.94 to 17.75°C (12.46 ± 2.26°C, mean ± 95% CI, $N = 8$), and the global model estimated the grass-line as 14.6°C. The higher sensitivity and specificity values for individual predictors were 0.99 and 0.73, both for annual mean temperature. Annual precipitation specificity had the lowest ability to predict C_3 or C_4-dominated sites, with sensitivity and specificity of 0.5 and 0.47, respectively. Multiple-factor models recovered significant coefficients ($p < 0.05$) for annual mean temperature and annual precipitation (**Supplementary Material 6**). Annual mean temperature showed a higher magnitude of changes in the C_3

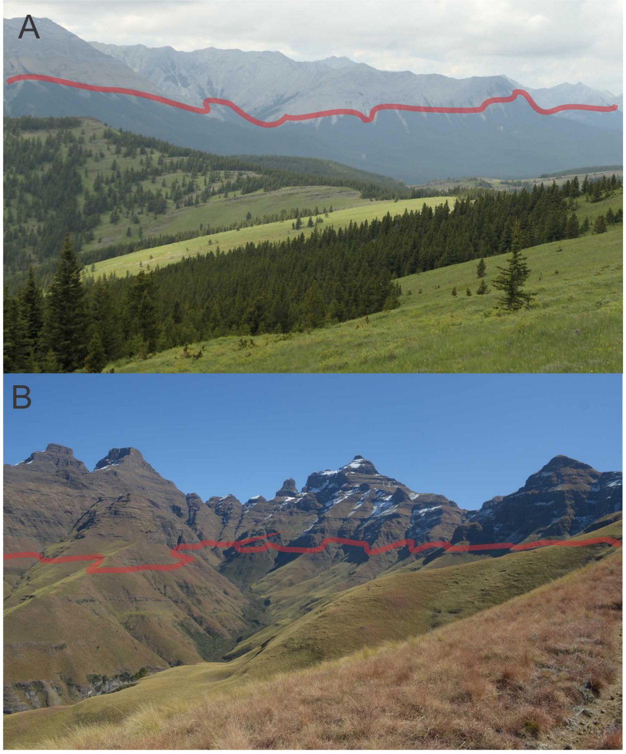

FIGURE 3 | Temperature-related ecological transition zones occurring on mountains. **(A)** Tree-line at c. 2,100 m on Compression Ridge in the Canadian Rockies. **(B)** C_3-C_4 grass-line transition zone at c. 2,500 m on Cathedral Ridge in the Maloti–Drakensberg (Cathedral Peak range, KwaZulu-Natal, South Africa). Pictures taken by S. D. Johnson.

proportion and a negative coefficient (−0.1061), while the lowest coefficients were identified for elevation (−0.0004) and annual precipitation (0.0003). The compositional shift between C_3-C_4 in the communities (that is, >50% of the recorded genera showing C_3 photosynthesis) was identified by the multiple-factor models in sites where the mean annual temperature gets lower than 14.6°C (**Figure 4**). Other predictors showed more variable effects.

C_3-C_4 Turnover in African Mountain Grasslands

In the African subtropical region, C_3 genera represent a considerable proportion of the taxonomic diversity in the southernmost mountains (32°S to 28°S), with at least 27 genera found in the Cape Midlands Escarpments (vs. 33 C_4) and 32 in the Maloti-Drakensberg (vs. 49 C_4). In the Cape Midlands Escarpments, C_3 genera become more abundant than C_4 genera above 1600–1700 m, which is the upper limit of the distribution of lowland C_4 genera like *Alloteropsis*, *Hemarthria*, and *Setaria*. Above 2200 meters, the only C_4 genera found are *Cymbopogon, Tetrachne,* and *Pennisetum*, while C_3 genera like *Helictotrichon* and *Festuca* reach elevations up to 2400 meters. In this C_3-C_4 shift region between 1600–1700 m, mean values for annual mean temperature was 12.9°C and for annual precipitation, 749 mm. In the Maloti-Drakensberg, the elevation identified for the community taxonomic transition between C_3 and C_4 genera was 2650 m, where annual mean temperature is ca. 9°C. C_3 genera like *Koordersiochloa*, *Stipa*, *Styppeiochloa,* and *Lamarckia* were only recorded above 2700 m, while many lowland abundant C_4 genera (*Brachiaria*, *Cynodon*, *Digitaria*, *Eleusine*, *Enneapogon*, *Hyparrhenia*, *Microchloa*, *Pennisetum, Setaria*, *Trachypogon*, *Tragus*) reach their upper elevational limit around 2850 m a.s.l.

Between 28°S and 10°S, the richness of C_3 genera decreases with the increase in temperature along the latitudinal gradient, while the number of C_4 genera increases. In the Mpumalanga-Limpopo mountains (26.7 to 24.7°S), for instance, we found 69 C_4 genera and 20 C_3 genera. Further north, between 20°S and 17.7°S, we listed 62 C_4 genera and 18 C_3 genera for Manica Highlands. For Mpumalanga-Limpopo, while the compositional shift gradually occurs as the temperature decreases along the elevation gradient, the grass-line is higher than the maximum elevation of the mountains, meaning that the proportion of C_3 genera does not surpass the proportion of C_4 at any point. The highest peak of Mpumalanga-Limpopo is 2319 m a.s.l., where annual mean temperature is 12.3°C and minimum annual temperature is 5°C. Around 33% of the Poaceae genera occurring at this elevation are C_3, which become gradually more common above 1500 m a.s.l., where genera like *Poa*, *Bromus*, and *Festuca* have their lower elevational boundaries. Meanwhile, several C_4 genera occur along the entire temperature gradient, like *Bothriochloa*, *Dactyloctenium*, *Paspalum*, *Schizachyrium*, *Aristida*, *Cenchrus*, *Trachypogon*, and *Eleusine*. On the other hand, Manica Highlands and Western Angola Escarpments are dominated by C_4 grasses (62 genera in Manica Highlands and 44 genera in Angola). Only two C_3 genera are recorded in Western Angola Escarpments, mostly at colder temperature sites found in high elevation belts (above 2050 m a.s.l.): *Isachne* and *Sacciolepis*. Even above 2050 m, the annual mean temperature ranges from 23.3 to 15.3°C, the minimum annual temperature ranges from 4.6 to 13.6°C, and the average annual precipitation is 1115 mm. For Manica Highlands, annual mean temperatures range from 12.9 to 17.84°C, and the average annual precipitation is 1354 mm. Since *p*-values calculated by individual models were higher than the significance threshold of 0.05 for both of these mountains, we removed them from the global model calculations and discussed their results individually.

C_3-C_4 Turnover in South America Mountain Grasslands

Grassland community gradient studies were available for Argentina (Mount Córdoba; Cabido et al., 1997) and Colombia (Transecto Sumapaz; Giraldo-Canas, 2010), and were included in our analysis. As expected, the proportion of C_3 genera increased in both areas as temperature decreases along the elevation gradient. In the Colombian Andes, the grass-line if found around 1,883 m a.s.l., where C_3 genera like *Arthrostylidium*, *Aulonemia*, *Avena*, *Calamagrostis*, *Danthonia*, *Festuca*, *Glyceria*, *Jarava*, *Nassella*, *Phalaris*, *Piptochaetium*, and *Rhipidocladum*

TABLE 2 | Individual mountains grass-line elevation and model coefficients for each generalized linear model fitted for C_3 proportion and elevation data.

Mountain	Grass-line temperature (°C)	Grass-line elevation (m)		Estimate	Std. Error	*z* value	Pr(> | z|)
Angola**	-	-	intercept	−6.743	3.587	−1.880	0.060
			coefficient	0.200	0.188	1.064	0.287
Cape Midlands	12.91	1608	intercept	4.145	1.136	3.648	0.000
			coefficient	−0.321	0.092	−3.487	0.000
Maloti-Drakensberg	8.94	2649	intercept	1.480	0.256	5.791	0.000
			coefficient	−0.166	0.023	−7.096	0.000
Manica Highlands**	-	-	intercept	−0.965	1.011	−0.954	0.340
			coefficient	−0.003	0.067	−0.043	0.966
Mpumalanga Limpopo*	10.28	2570	intercept	2.549	1.045	2.440	0.015
			coefficient	−0.248	0.070	−3.555	0.000
Mount Córdoba	11.44	1813	intercept	5.194	0.554	9.369	0.000
			coefficient	−0.454	0.042	−10.749	0.000
Sumapaz	17.75	1883	intercept	2.578	0.134	19.247	0.000
			coefficient	−0.145	0.007	−20.502	0.000
Mongolian Mounts*	9.77	839	intercept	3.176	0.859	3.698	0.000
			coefficient	−0.325	0.151	−2.154	0.031
New Guinea	16.95	2024	intercept	2.336	0.129	18.096	0.000
			coefficient	−0.138	0.007	−19.962	0.000
New Zealand*	11.62	−103	intercept	7.696	0.814	9.459	0.000
			coefficient	−0.662	0.088	−7.559	0.000

**Sites where grass-line modeled values fall beyond the current climatic conditions and elevation range of the mountain. **Sites where we recorded a weak effect of temperature or elevation on C_3 proportion ($p > 0.05$).*

TABLE 3 | Single-factor models coefficients, intercepts, identified threshold for higher than 50% C_3 genera composition, sensitivity, specificity, and associated *z*-value probabilities.

Predictor	Model coefficient	Model intercept	Modeled threshold	Sensitivity	Specificity	Significance
Annual mean temperature (°C)	−0.1208	1.7610	14.6°C	0.9917	0.7253	$p < 0.001$
Maximum temperature in the warmest month (°C)	−0.1577	3.6735	23.3°C	0.7711	0.7059	$p < 0.001$
Annual mean precipitation (mm)	0.0001	−0.223	2589 mm	0.7397	0.6377	$p < 0.001$

have the lower elevation limits of their distribution. For this region, we calculated an annual mean temperature of 20.2°C, a minimum annual temperature of 14.7°C, and an annual precipitation of 1550 mm. For Patagonia and the Southern Andes in Argentina, four genera of C_3 grasses (*Agrostis*, *Panicum*, *Poa*, and *Vulpia*) had their lower distribution limits around 1400 m, while C_4 genera become gradually less abundant, since genera like *Andropogon*, *Axonopus*, *Gymnopogon*, and *Elionurus* have their upper elevation limit around 1550 meters. In this region, the grass-line in Mount Córdoba is found in 1813 m, where the annual mean temperature is 13°C, minimum annual temperature −7.1°C, and annual precipitation 617 mm.

C_3-C_4 Turnover in Asian Mountain Grasslands

In the temperate Mongolian Mountains, 11 C_4 genera were found, with some occurring along the entire elevation gradient of the mountain, such as *Cenchrus*, *Cleistogenes*, and *Setaria*. There are 18 C_3 genera recorded and some are found even in low elevation sites, like *Agropyron*, *Poa*, *Elymus,* and others. Annual mean temperatures range from 3 to 7.4°C, with maximum temperatures in the warmest month ranging from 25 to 29.3°C. In comparison, tropical mountains of Asia, like New Guinea, show a transition between C_3 and C_4 along the elevation gradient. The C_3 turnover is observed around 2050 m a.s.l., where the average annual mean temperature is around 17°C. Overall, we listed 58 C_3 genera and 64 C_4 genera for the mountains. Most C_3 genera (26 of 58) have their lower distribution limits around 2750 m (e.g., *Anthoxanthum*, *Brachypodium*, *Cyperochloa*, *Danthonia*, *Dichelachne*, *Isachne*, and *Trisetum*), and only six C_4 genera have records above 2800 m (*Arthraxon*, *Arundinella*, *Capillipedium*, *Coix*, *Digitaria*, and *Dimeria*).

C_3–C_4 Turnover in Australia and New Zealand

In the New Zealand Mountains, we listed 45 C_3 and 14 C_4 genera, and identified occurrences of C_4 genera like *Digitaria*, *Cenchrus, Echinochloa*, and others in warmer sites, in elevations bellow 1050 m, but mountains are almost entirely dominated by C_3 genera. The dominant climate in New Zealand mountains is Temperate

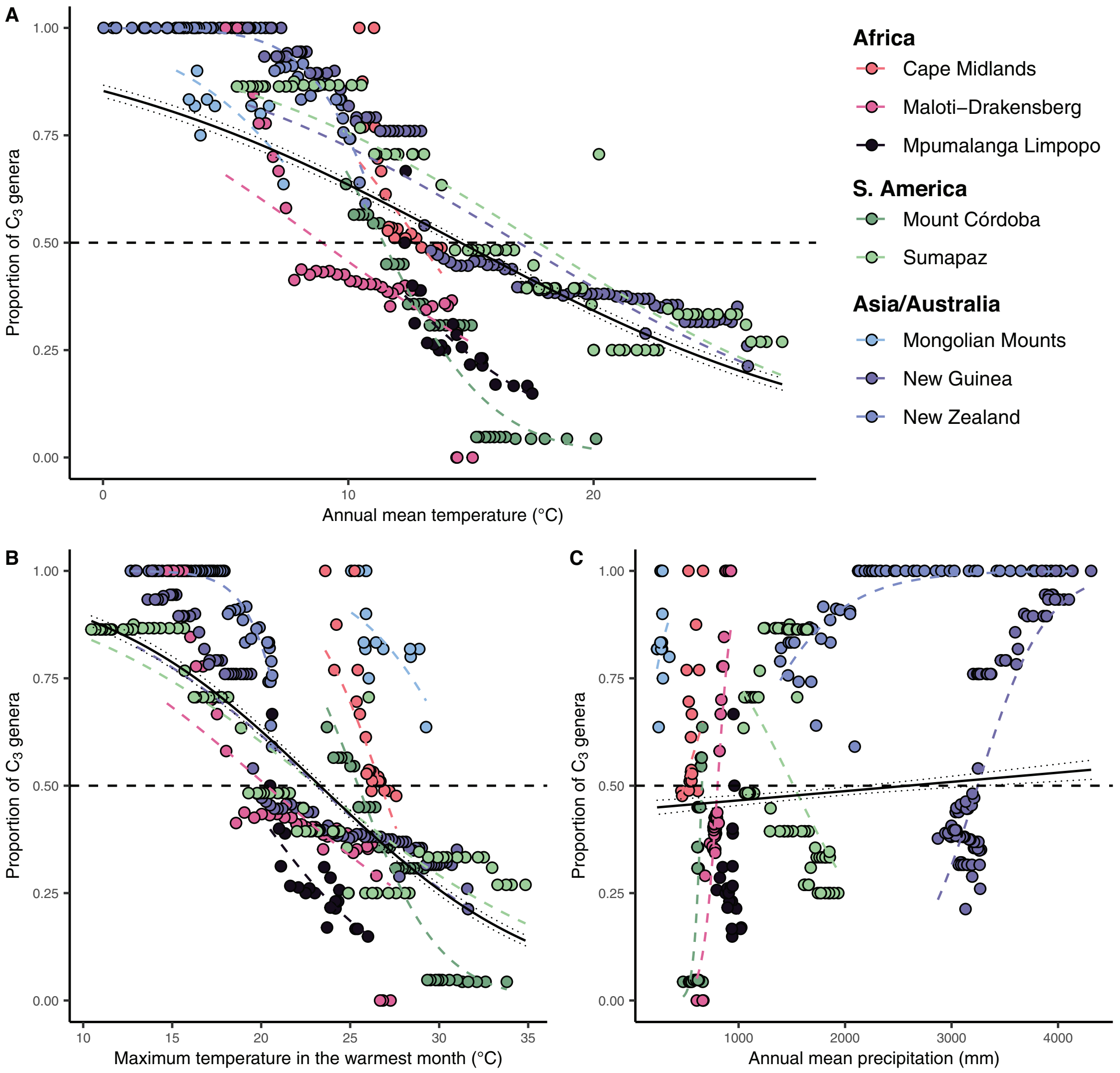

FIGURE 4 | The global relationship between the proportion of C_3 genera in each elevation interval and their respective maximum temperature in the warmest month and annual precipitation. **(A)** Annual mean temperature (°C) vs. the proportion of C_3 Poaceae genera in each elevation interval. **(B)** Maximum annual temperature (°C) vs. the proportion of C_3 Poaceae genera in each elevation interval. **(C)** Annual precipitation (mm) vs. the proportion of C_3 Poaceae genera in each elevation interval. Each point represents a 50 m elevation belt in each mountain (represented by the colors on the legend) in each continent/region. Dashed colored lines represent individual mountains fitted logit link function with a binomial distribution (with events/trials structure) for the number of C_3 and C_4 genera found in each site. The continuous black lines represent the global fitted logit function for each variable with 95% confidence interval. The horizontal dashed black line represents the 50% of C_3 threshold, proposed here as the grass-line.

oceanic (Cfb), with annual mean temperatures ranging from 0 to 10.7°C.

DISCUSSION

The Transition Between C_3 and C_4 in Grassy Mountains Is Driven Mainly by Temperature

The turnover from C_4 to C_3 dominated grasslands with decreasing temperature along the elevation gradient is transitional and is not in the form of a sharply demarcated line (**Figure 3**). It occurs along an isotherm, a temperature-linked elevation interval, which has a stronger effect than precipitation and elevation itself. The same phenomenon occurs with tree-lines, which are very often a transitional zone distributed over hundreds of meters. Transitional change does not mean that the grass-line cannot be used for monitoring as logistic regression models can provide temperatures and elevations associated with probabilities of turnover from C_4 to C_3 dominated grasslands. Based on our results, we argue that identifying the elevation interval where the annual mean temperature is around 14.6°C is indicative of the elevation range where C_3 genera become more representative than C_4, especially for subtropical grassy mountains. While there is a range of variation in the grass-line temperature for empirical datasets, the average value of 14.6°C can be used as an initial reference to the development of locally suited plots focused on tracking changes in the "grass-line" over time, since the overall pattern is effectively demonstrated: lower temperature sites show higher probabilities of being dominated by C_3 genera. Here, we also present a model of the general guidelines for the development of such protocols using the Maloti-Drakensberg as an example.

Several factors like phenotypic plasticity, C_4 acclimatization, microclimatic variation, edaphic factors, biologic interactions, regional genera pool, biogeographic histories of individual mountains, and even methodological limitations (spatial resolution of the mountain delimitation polygons, climatic datasets, and digital elevation models) could influence the results observed for Angola and Manica Highlands, and affect the precision of the global model. Thus, the global model is a starting point to the development of individual mountain-focused protocols, and elevation intervals need to be locally identified and adapted to be effectively applied for the development of locally suitable plot designs to track the changes in C_3-C_4 composition over time and the point of inflection where the communities along the elevation gradient begin to become C_3 dominated. It is important to replicate our analysis in individual sites and test for further convergence of temperatures linked to the grass-line in each mountain before applying the protocol. This is necessary since our results are limited by sample size, as grass-lines are physically present in five of the ten mountains we analyzed. Even for modeled grass-lines, like the ones we identified for Mpumalanga Limpopo, Mongolian Mountains, and New Zealand, there was a considerable variation in annual mean temperature thresholds, suggesting that the grass-line may be a more suitable tool for certain localities than others (like Manica Highlands and Angola, for example). For these tropical mountains where the transition was not empirically observed, the combination of warm temperatures and a limited regional pool of C_3 genera (only 2 of 45 Poaceae genera found in Angola are C_3) justifies the low effect of annual temperature on the increase of C_3 in Angola. On the other hand, the tropical conditions and the relative lower elevation extent (2558 m) in Manica Highlands, when compared to other tropical mountains included in our analysis (4613 m in New Guinea and 4200 m in Mount Sumapaz) do not allow for the temperature gradient to reach the same temperate conditions observed for higher elevation belts of other tropical mountains. This result suggests that the grass-line is more efficient for subtropical mountains, which already have a local pool of C_3 species and where the elevation gradient reaches temperatures lower than the global threshold of 14.6°C.

This C_3-C_4 grass-line has a dynamic temporal history, with isotope reconstructions indicating that past climates greatly affected C_3-C_4 proportional dominance globally; data indicate a pronounced increase in C_4 dominance for both sub-tropical and tropical regions during cold and dry periods, like the Last Glacial Maximum (Ehleringer et al., 1997; Collatz et al., 1998; Osborne, 2008; Edwards et al., 2010). Controversially, available data suggest that even though C_3 grasses might have historically benefited from a carbon-richer atmosphere over C_4 species (Ehleringer et al., 1997; Collatz et al., 1998; Osborne, 2008; Edwards et al., 2010), temperature is still one of the overriding determinants of their dominance (Collatz et al., 1998; Edwards and Still, 2008). Even though the evolution of C_4 grasses seems to be strongly influenced by warmer climatic conditions (Sage, 2001; Sage et al., 2018), in an evolutionary context, however, temperature change itself is not capable of explaining C_4 plant evolution (Sage, 2004). Instead, it is likely that complex interactions between biological, atmospheric, and geologic elements interacted (Sage, 2004). Interestingly, even though current CO_2 levels are rising dramatically, C_3 plants still employ substantial metabolic effort in sustaining a photosynthetic apparatus capable of handling low CO_2 concentrations (Sage and Coleman, 2001).

This pattern is a direct consequence of the relatively poor photosynthetic performance of C_3 plants under warm climatic conditions, a well known as physiological limitation caused by the lower specificity of RuBisCO under such conditions, resulting in a metabolic waste of energy in the form of photorespiration. As a result, in cooler mountains (such as New Zealand and northern China), the whole elevation continuum is dominated by C_3 genera, while warmer tropical mountains (such as the Angolan Highlands) are dominated by C_4 genera (**Figure 2**). For example, in New Zealand, while there are occurrences of C_4 genera at warmer belts found in low elevations (below 1050 m), all the upper belts are dominated by C_3 genera, while in China given the remarked temperate climate found in these mountains, most elevation belts present a relatively stable 70% C_3 composition, with no clear increase along the elevation gradient (**Supplementary Material 7**). Even in tropical mountains like the Northern Andes, where the climatic heterogeneity is considerably higher (Rahbek et al., 2019a), C_3 genera like *Alopecurus*, *Aphanelytrum*, *Brachypodium*, *Cinna*, *Cortaderia*, *Elymus*, *Neurolepis, Phleum*, *Ortachne*, *Triniochloa*, and *Trisetum* only become dominant above 2450 m a.s.l., where the mean annual temperatures become lower than 14.6°C. It is noteworthy though, that air temperature *per se*, despite being highly correlated, is not a direct measure of leaf temperature for low stature functional groups, which is usually warmer than the atmospheric measure because of boundary layer effects. Also, since WorldClim variables are interpolated 2 m above ground-level weather-station data, we recognize that our temperature data is possibly not refined enough to capture more local scale dynamics. In mountain systems where there is snowfall, grasses might also be periodically isolated from atmospheric conditions whenever there is snow accumulation. For trees, this is not observed, and it is one of the reasons for the sharper tree-line limit observed in forest-dominated mountains (Körner, 2003; Scherrer and Köerner, 2010; Scherrer and Körner, 2011).

As the World's grassy mountain systems are most extensive in the moisture-stressed mid-latitudes, moisture availability (or moisture reliability) may favor C_3 over C_4 richness in these regions. However, precipitation itself seems to play a weak role (**Figure 4** and **Supplementary Table 3**) in this community shift at the local gradient level, even though it may play an important role in explaining variation among regions (Edwards and Still, 2008) and also the global dominance of C_4

grasses (Adie et al., 2017). Our models therefore reinforce the role of temperature as the primary driver of the C_3-C_4 "grass-line," in the same way that it determines the global distribution of C_3 and C_4 grass communities, surpassing even the effects of the latitudinal gradient.

One immediate implication is that—with temperature increases of 1.5°C projected for the next 50 years under current emission scenarios (Hock et al., 2019)—as the C_3-C_4 threshold shifts upslope under warmer temperatures, montane C_3 grasses are likely to undergo contractions in their distributions. Narrow endemic genera (like *Arthrostylidium*, *Aulonemia*, *Calotropis*, *Danthonia*, *Deyeuxia*, *Rytidosperma*, *Sacciolepis*, *Trichopteryx*, and *Vulpia*) may be at most risk from population bottlenecks and a decrease in photosynthetic productivity, or even local extinction. Meanwhile, C_3 dominated mountains (e.g., temperate mountains in New Zealand and China) will face an increase in the presence and dominance of C_4 species and higher vulnerability to invasions of alien C_4 grasses. Thus, biodiversity erosion will certainly be a consequence of increased temperatures in currently C_3-dominated mountains, due to the predicted warming of originally colder sites found in higher elevation in other sub-tropical mountains.

Developing a Grass-Line Protocol: How Can we Track the Transition in Grassy Mountains?

While developing strategies to track the grass-line is challenging and needs to be continuously developed, we outline some basics for such study designs when monitoring grassy mountains. Thus, the geographic distribution and the sizes of the plots—ideally large permanent rectangular plots with randomly distributed smaller sampling plots—need to be carefully planned and evaluated for each region.

As a case in point, we hereby propose a pilot protocol to monitor C_3-C_4 grass community shifts in grassy mountains using the Maloti-Drakensberg as an example. We propose a permanent nested-plot-based design that can be revisited every 2–5 years, with more frequent surveys during years of environmental extremes. Permanent plots should span the whole ecotone (i.e., cover an elevational gradient), centered approximately on the grass-line calculated for a given mountain. Target areas can be delimited using GIS tools to maximize the inclusion of grassland transects known to include both C_3 and C_4 genera, where multiple permanent larger rectangular plots (e.g., 100 × 50 m) are established along the elevation gradient with smaller 1 × 1 m subplots systematically sampled to measure presence/absence for indicative grass genera (**Figure 5**). With this data, it becomes possible to calculate the relative abundance of C_3 vs. C_4 grasses for the large plots and to monitor shifts in the distribution of indicative genera along the elevational gradient (in the Maloti-Drakensberg the transition would typically start around 1850–2000 m in elevation, reaching 50% around 2650 m). Additionally, this protocol would allow for monitoring the occurrence and abundance of native and invasive C_4 genera above the predicted elevational threshold over time, as well as monitoring population declines in indicator C_3 genera occurring in high-elevation sites (e.g., above 1850 m a. s. l. in the Maloti-Drakensberg). By monitoring the "grass-line" over time, while simultaneously tracking ongoing temperature changes, it will be possible to estimate the habitat loss for C_3 genera, the rate of expansion of C_4 genera, and, as a result, the increasing vulnerability of grass-dominated mountain peaks to lowland taxa (as reported for tree-dominated temperate mountains by Steinbauer et al., 2018). Overall, the protocol needs to take into account the individual environmental characteristics of each region and the diversity of genera found in each elevation associated with the transition to be able to capture changes in this composition over time.

The grass-line is complementary to monitoring the elevational limits of individual species, but it is preferred since it provides a generalized approach. It uses a functional/physiological metric that is less affected by, for example, non-thermal distributional boundaries of native species which may be limited to specific geologies or soil moisture levels and will not respond to changes in temperature; or the establishment of alien species, which may cause big changes in species composition, but are unrelated to changes in climate. The grass-line offers the same advantage as (other) functional trait approaches—it allows us to compare areas irrespective of species composition, using a functional/physiological metric that has direct ecological relevance.

Under changing environmental conditions we expect to see some degree of idiosyncrasy in the responses of individual species, and using a "community-level" metric will provide a better indicator of how the ecosystem is changing. As in tree-line studies, "grass-line" ecotone behavior is best monitored through tracking the growth and movement of C_3 and C_3 genera/species in fixed plots, resampled at intervals. A similar protocol is the Mountain Invasion Research Network (MIREN) that determines the behavior of non-native species in mountains. By designing a protocol that can be replicated across the grass-line ecotone, and repeatedly sampled at temporal intervals we can determine the behavior of the grass-line on a large scale.

Future Climatic Scenarios Impact on This Composition Shift and the Importance of a Grass Focused Protocol

The social-ecological impacts of shifts in the C_3-C_4 "grass-line" on culture, livelihoods, and rangeland-dependent food security will be likely considerable, and will require major adaptations in both (1) fire management—given that higher temperatures favor fire-prone C_4 grasses over fire-retarding C_3 grasses (Clark et al., 2009)—and (2) grazing governance—as natural pasture palatability will change in response to grass community changes; grazing management will need to adapt accordingly to become sustainable (Clark et al., 2009). The currently available evidence is not conclusive about quantitative implications of the C_4 to C_3 shifts in the cascading ecological effects of this community transition and its net impact on herbivores (Chamaillé-Jammes and Bond, 2010). Therefore, developing a protocol monitoring grassland community shifts in C_3-C_4 generic composition offers a valuable tool to quantify and to identify sites where this shift may occur. Moreover, monitoring "grass-line" boundaries can clarify

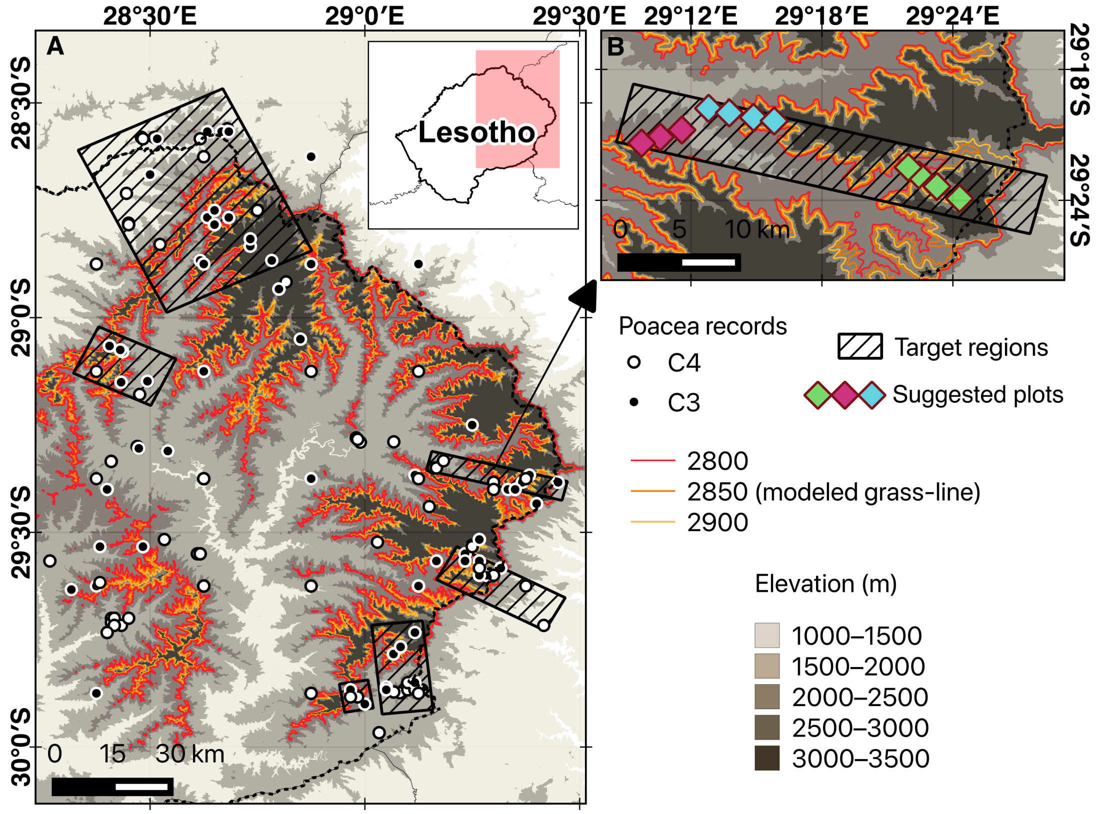

FIGURE 5 | Example of site selection for grass-line large and small plots using the Maloti-Drakensberg as a point in case.

the real impacts of regional community changes in phenology, digestibility, and productivity, since they may vary substantially between C_3 and C_4 grasses, and these traits are likely to affect the diets of large mammalian herbivores (Ehleringer et al., 2002). Among these groups, productivity is also a complex function influenced by multiple environmental conditions (e.g., daytime growing season temperature, nitrogen, water, carbon), making it difficult to establish a generalizable differentiation and to predict precisely expected local changes, reinforcing the necessity to monitor and measure this shift at regional scales (Chamaillé-Jammes and Bond, 2010). There will likely be both "winners" and "losers" from such changes. For example, longer growing seasons may increase carry capacity in some regions (provided aridity does not also increase), and many C_4 grasses may be palatable than C_3 grasses (e.g., C_4 *Themeda* over C_3 *Tenaxia* in southern Africa) (Clark et al., 2009) suggesting a switch to more favorable natural pastures. All these peculiarities reinforce the need for monitoring this change in multiple scales across different environments, and the "grass-line" concept provides a suitable protocol for this purpose.

For many of the World's mountains there is evidence that sustained increases in temperature over the past century and land-use related disturbances have promoted upward elevation shifts in the tree-line (Ameztegui et al., 2016; Cazzolla Gatti et al., 2019) as well as increasing incidence of "lowland" taxa at novel higher elevations, with standardized global protocols being used to track these ecological responses (Grabherr et al., 2000; Naccarella et al., 2020). Historical compositional shift datasets are available to many temperature mountains (mostly in western Europe), and they provide important evidences of how climate change is affecting mountains biodiversity and how conservation efforts can be directed to mitigate these impacts (Steinbauer et al., 2018). At present, there are no comparable data for grassy mountain systems, especially in the southern hemisphere. We suggest that a common global protocol to monitor the C_3-C_4 "grass-line" in grassy mountain systems may provide equivalent data for tracking change and providing scientific support for climate adaptation programs in such mountains.

DATA AVAILABILITY STATEMENT

The datasets are available as **Supplementary Material**.

AUTHOR CONTRIBUTIONS

JD developed the methodology, collected and analyzed the data, prepared the figures, developed the protocol, and wrote the foundation manuscript and supplementary data. PR provided the data, developed the protocol, and assisted with constructing the body text. SJ assisted with data analysis and figures, and edited the manuscript. MB assisted on the discussion of the results, developed the protocol, and edited the manuscript. VC conceived the study, collected and provided field data, assisted with constructing the body text, and provided project leadership. All authors contributed to the article and approved the submitted version.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.760118/full#supplementary-material

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Evolutionary and Ecological Explanations for the Elevational Flexibility of Several East African Bird Species Complexes

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Africa's montane areas are broken up into several large and small units, each isolated as forest-capped "sky islands" in a "sea" of dry lowland savanna. Many elements of their biota, including montane forest birds, are shared across several disjunct mountains, yet it has been difficult to rigorously define an Afromontane forest avifauna, or determine its evolutionary relationships with the birds of the surrounding lowland forests. In order to trace the historical relationship between lowland and highland avifaunas, we review cases of species or groups of closely related species with breeding populations at different elevations, and use phylogeographic methods to explore the historical connections between such populations within the biodiversity hotspot of East Africa. The study reveals several idiosyncratic patterns, but also a prominent number of cases of gene flow between populations in southern areas, mainly around the Malawi Rift, and mountains and coastal forests to the north, close to the equator. This may reflect more continuous past distributions through northern Mozambique and coastal Tanzania, or seasonal migrations between areas with different rainfall regimes. Over time, these distributional dynamics have resulted in a higher persistence of lineages, and an accumulation of forest-dependent lineages within the Eastern Arc Mountains of Tanzania and the northern part of the coastal forest mosaic.

Keywords: hotspot, birds, phylogeography, dispersal, corridors

INTRODUCTION

The Afromontane region comprises punctuated chains of mountains, which mostly follow the East African rift systems, and are characterized by a distinct botanical assemblage in areas above 1,500–2,000 m in elevation (White, 1981). This mountainous region comprises volcanoes as well as uplifted enclaves of ancient crystalline bedrock extending from: (1) the Ethiopian Highlands, through (2) the Kenyan Highlands and northern Tanzania, (3) East Congo/Albertine Rift, (4) the Eastern Arc Mountains, which run from southeastern Kenya diagonally across Tanzania, to (5) the highlands of Malawi, continuing through the Chimanimani Mountains of Zimbabwe/Mozambique to South Africa, with the (6) Cameroon Highlands and (7) Angolan Highlands as isolated montane areas near the west coast of the continent. Each of these montane areas are themselves fragmented into small and large mountain blocks isolated in a "sea" of lowland savanna, and these montane areas are often referred to as "islands in the sky," an archipelago of montane habitat within the larger expanse of the African continent. The archipelago-like distributions of mountains within and among African montane areas of endemism, differ fundamentally from other large and biologically diverse montane systems such as the Himalayas, Andes, or Rocky Mountains, where elevational bands of uniform vegetation have much greater linear continuity along the mountain range.

In eastern Africa, approximately half of all species of forest birds are confined to evergreen montane forests, which are generally recognized as a distinct ecological zone from the semi-deciduous forests of the coastal zone, known as the "Zanzibar-Inhambane coastal forest mosaic." In spite of this, attempts to divide Africa into biochoria with distinct biota, have failed to identify a distinct area unit for Afromontane birds (Diamond and Hamilton, 1980; Crowe and Crowe, 1982; deKlerk et al., 2002; Linder et al., 2012; Holt et al., 2013). This is primarily because the small and patchy distribution of many Afromontane species and the high turnover across sites provide little connectivity in cluster analyses. Further, the rather coarse geographical grid (often 1° squares) that is typically used in such analyses, includes a greater number of species from the non-montane habitat matrix, which creates greater statistical connectivity with the surrounding landscapes (deKlerk et al., 2002; Linder et al., 2012). While only some 15% of the forest-associated birds of eastern Africa are endemic to the coastal forests, most other non-montane forest birds are quite widespread, occurring wherever there are patches of semi-evergreen vegetation on floodplains and in the foothills of montane highlands. Finally, some African bird species are patchily distributed both in highland and lowland habitats (e.g., East Coast Akalat *Sheppardia gunningi*, Fjeldså et al., 2000), and several birds of montane forests are phylogenetically nested within clades of lowland birds (and *vice versa*), suggesting dynamic shifts between lowland- and highland-breeding. The boundary between the lowland and highland avifaunas appears therefore to be fuzzy, and contributes to making biogeographic subdivisions for African birds challenging (Dowsett, 1986; Bowie, 2003).

To date, most phylogeographic studies of Afromontane birds have focused on discrete groups of taxa that diversified across the described mountain regions. In this article, we focus instead on species with mixed elevational distributions to explore the variation in distribution patterns and search for historical links between populations breeding in cool highland forests and semi-evergreen habitats in the hot lowlands. We also try to determine whether flexibility in elevational distribution is associated with specific ecologies. We restrict our study to the Tanzania-Malawi Rift Mountains and the adjacent coastal forest mosaic, which together constitute the "Eastern Afromontane Biodiversity Hotspot" (Mittermeier et al., 2004), where high species diversity has accumulated in mountains where forests persisted – likely on a permanent basis – since before the break-up of the Pan-African rainforest in the Miocene, when large parts of Africa changed to become dominated by savanna and mixed-woodlands (Lovett and Wasser, 1993).

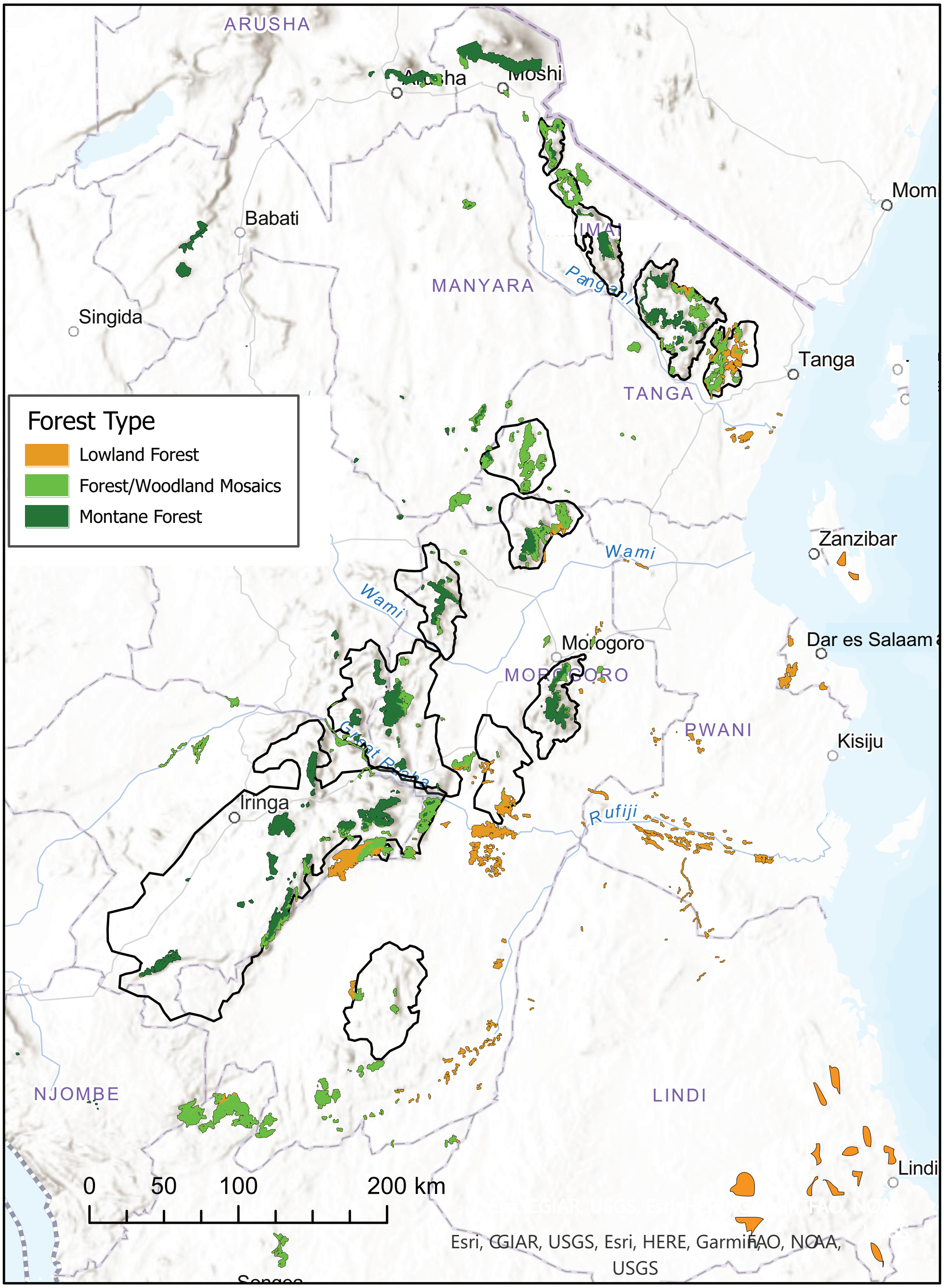
We describe cases of bird species, or groups of closely related species, which comprise distinctive populations of breeding individuals that occupy both highland and lowland forest habitats, as well as montane species, whose present distributions seem to indicate past historical connections across lowland areas. Based on the diversity of observed distribution patterns of birds in the Eastern Afromontane Biodiversity Hotspot, we aim to address the following questions: (1) Are disjunct elevational distributions of African bird species a rare anomaly, or can we find recurring patterns, and if so, (2) how can we explain the shifts in ecology that must have taken place? (3) Is there a specific evolutionary history that underpins the basis of joint lowland and highland residency across a species range, or are there some common ecological factors or life history traits (e.g., canopy versus understorey feeding) that are not directly linked with elevation?

We revisit published phylogeographic studies of African montane and lowland bird species and combine these results with summaries of additional case studies from the literature. We synthesize these data and use the results to discuss the possible origins of disjunct elevational distribution of some African bird species in relation to climate history and a putative common ecological cause.

STUDY REGION AND DATA

The Eastern Afromontane Biodiversity Hotspot represents a suitable area for studying these questions because of its complex landscapes (**Figure 1**), where isolated mountains and punctuated chains of montane fault-blocks with patches of montane rainforest occur in a matrix of hot and dry lowland savanna with local patches of semi-evergreen forest in drainage seeps and around springs. This can be in the form of foothill forests, groundwater forests associated with the major floodplains, or forest patches near the coast toward the Indian Ocean (**Figure 1**). While mountains of Kenya and northern Tanzania, and those along the Malawi Rift, are relatively young and partly of volcanic origin, the Eastern Arc Mountains, which run diagonally across Tanzania from the south-west to the Taita Hills in south-eastern Kenya, consist of ancient basement rock that was uplifted over a long period of time, with the final uplift in the late Miocene (>7 million years ago; Griffiths, 1993). Patches of montane rainforests in the Eastern Arc have been interpreted as remnants of the ancient trans-African super-rainforest (Axelrod and Raven, 1978; Lovett, 1993), which broke up during the late Miocene as a consequence of uplift in central Africa, global cooling (deMenocal, 2004), and a shift to more grass-dominated ecosystems over much of Africa (Vrba et al., 1995; Jacobs et al., 1999; Strömberg, 2011).

The Eastern Arc Mountains owe their high biodiversity to a predictable supply of humidity from the Indian Ocean, which presumably has been constant since the Miocene (Prell et al., 1980). The eastern escarpments have high orographic rainfall, mainly from November through April. In the East Usambara



Esri, CGIAR, USGS, Esri, HERE, Garmin, FAO, NOAA, USGS

FIGURE 1 | Map of montane and lowland forest fragments distributed across eastern Africa. Black polygons demarcate the boundaries of the Eastern Arc Mountains of Tanzania (see **Figure 7**). Data compiled as part of the CMEAMF baseline report [Forestry and Beekeeping Division (2006)].

Mountains, the high humidity from the ocean means that even low hills can maintain cloud forest (Lovett, 1993). The high plateaus further inland are often enshrouded by clouds, which help to maintain soil humidity and therefore the presence of evergreen forest. However, much of the highlands lack forest, because of human activities (burning and clearing for agriculture), or due to low heat-retention in small highlands and frost damage to the vegetation (Sarmiento, 1986; Lovett, 1993).

Changes in abiotic factors along the elevational gradient have resulted in distinct bands of vegetation (Lovett, 1993). Mountains along the Malawi Rift are influenced by a more local convection-rainfall, where the rainfall can be somewhat out of pace with the seasonal cycle from the Indian Ocean, and where the climate becomes generally cooler at more southerly latitudes.

The lowlands of East Africa are mainly characterized by savanna woodland and scrubland, with semi-evergreen aspects only locally, and especially along the coastal zone (Burgess and Clarke, 2000). The patches of coastal forest that remain today in the densely populated coastal zone are of quite variable appearance, with little green foliage in the dry season, except in places with high ground water levels near the major rivers, in higher-lying areas with special soils, or in places where fog may accumulate during the night. The understorey is typically dominated by *Elyra* grasses and has few ferns; screw-palms (*Pandanus*) can form distinct stands in places with high levels of ground water. There is a flush of green leaves, flowers and insect life during the rainy season, which extends from November to April, or as two annual peaks around equinox as we approach the equator in northern Tanzania and Kenya.

Intensive charting of the distribution of biodiversity has taken place since the 1990s, with comprehensive review of the literature and of material in major museums, and with recent ornithological surveys to virtually every tract of montane forest and also to many lowland forests in Tanzania. These efforts have been supplemented by other recent initiatives, and by bird atlas projects covering much of the region (Dowsett-Lemaire and Dowsett, 2006)[1], resulting in detailed distributional databases of species occurrences (Burgess et al., 2006; Rovero et al., 2014). Unfortunately, northern Mozambique is still poorly explored.

Tissue and blood samples of birds for genetic study have been collected in Tanzania and Malawi over the past 20 years by members of several institutions (see "Acknowledgments") and we make use of some of these samples in our present study.

The results reported in this study are based on Sanger sequencing of mitochondrial markers [NADH dehydrogenase subunit 2 (ND2) and subunit 3 (ND3), Cytochrome b (Cytb), ATP Synthase membrane subunit 6 (ATP6)] and several nuclear introns [e.g., Fibrinogen beta chain intron 5 (FGB5), Glyceraldehyde 3-phosphate dehydrogenase intron 11 (GAPDH 11), Transforming growth factor beta 2 intron 5 (TGFb2)] following standard methods (see Fuchs et al., 2004; Kimball et al., 2009; Bowie et al., 2018). Specific markers used are mentioned with respect to each case study. The loci were aligned using MAFFT (Katoh and Standley, 2013). Phylogenetic trees were built using parsimony in PAUP* (Swofford, 2002) and/or by using maximum likelihood via RAXML v8.2.12 (Stamatakis, 2014) as implemented through the CIPRES supercomputing portal (Miller et al., 2010) under a GTR model of nucleotide substitution; with support for nodes evaluated using bootstrapping. To estimate times of divergence we made use of the rates of molecular evolution calculated by Lerner et al. (2011) for the Hawaiian honeycreeper radiation that we implemented through use of a Bayesian algorithm in BEAST (Drummond and Rambaut, 2007).

RESULTS

We first review published phylogeographic studies of East African bird species with special focus on evolutionary relationships between highland and lowland populations, and for where signs of gene flow between highland and lowland populations have been postulated. In addition, we also mention additional examples of East African bird species whose distribution patterns are suggestive of similar shared histories between montane and lowland habitats, and note that these taxa are in need of phylogeographic analyses.

Cisticolidae, African Warblers: The *Artisornis*/*Oreolais* Lineage

The four species comprising the genera *Artisornis* and *Oreolais* are insectivorous warblers restricted to the vine-tangles and dense understorey vegetation of montane forests across east and central Africa (**Figure 2**, Nguembock et al., 2008; Bowie et al., 2018). The African Tailorbird *A. metopias* is distributed from northern Tanzania to the mountains on the eastern side of Lake Malawi in southern Tanzania (Matengo Highlands) and northern Mozambique (Serra Jeci, near the east bank of Lake Malawi). Its sister-species, the Long-billed Tailorbird *A. moreaui* has a strange, disjunct distribution with one population on Serra Jeci and another 1,000 km away, in the lower to middle montane forests of the East Usambara Mountains near the coast of northern Tanzania (**Figure 2**). This distribution pattern has puzzled many biogeographers (e.g., Stuart, 1981).

Bowie et al. (2018) provided evidence in support of the contemporaneous Pleistocene vicariance of the once continuous range of the African Tailorbird into four distinct clades each reciprocally monophyletic: (1) Usambara Mountains, (2) Central Eastern Arc Mountains, (3) Uluguru Mountains, and (4) the Matengo and Serra Jeci Highlands; **Figure 2**). The simultaneous separation of the two populations of *A. moreaui* with that of the divergence of the four populations of *A. metopias,* suggests that both species were affected at the same time during the Pleistocene, when Africa became more arid (deMenocal, 2004; Trauth et al., 2005; Lyons et al., 2015) and lowland and highland forests were fragmented. To explain the present disjunct distribution of the two extant populations of Long-billed Tailorbird, Bowie et al. (2018) inferred a direct dispersal via lowland forest between the East Usambara Mountains (one of the few places in Africa where montane and lowland forest about) and the forest habitats of northern Mozambique. Molecular data from other montane bird species (e.g., Bowie et al., 2006; Fjeldså et al., 2006; Fuchs et al., 2011) as well as montane frogs (Lawson, 2013; Portik et al., 2019) suggests the existence of such a lowland corridor along coastal Tanzania and possibly across the hilly landscape of southern Tanzania and northern Mozambique. Lowland forests are assumed to have been far more extensive in the past than they are today (Burgess and Clarke, 2000), and therefore *A. moreaui*

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[1] http://tanzaniabirdatlas.net/start.htm

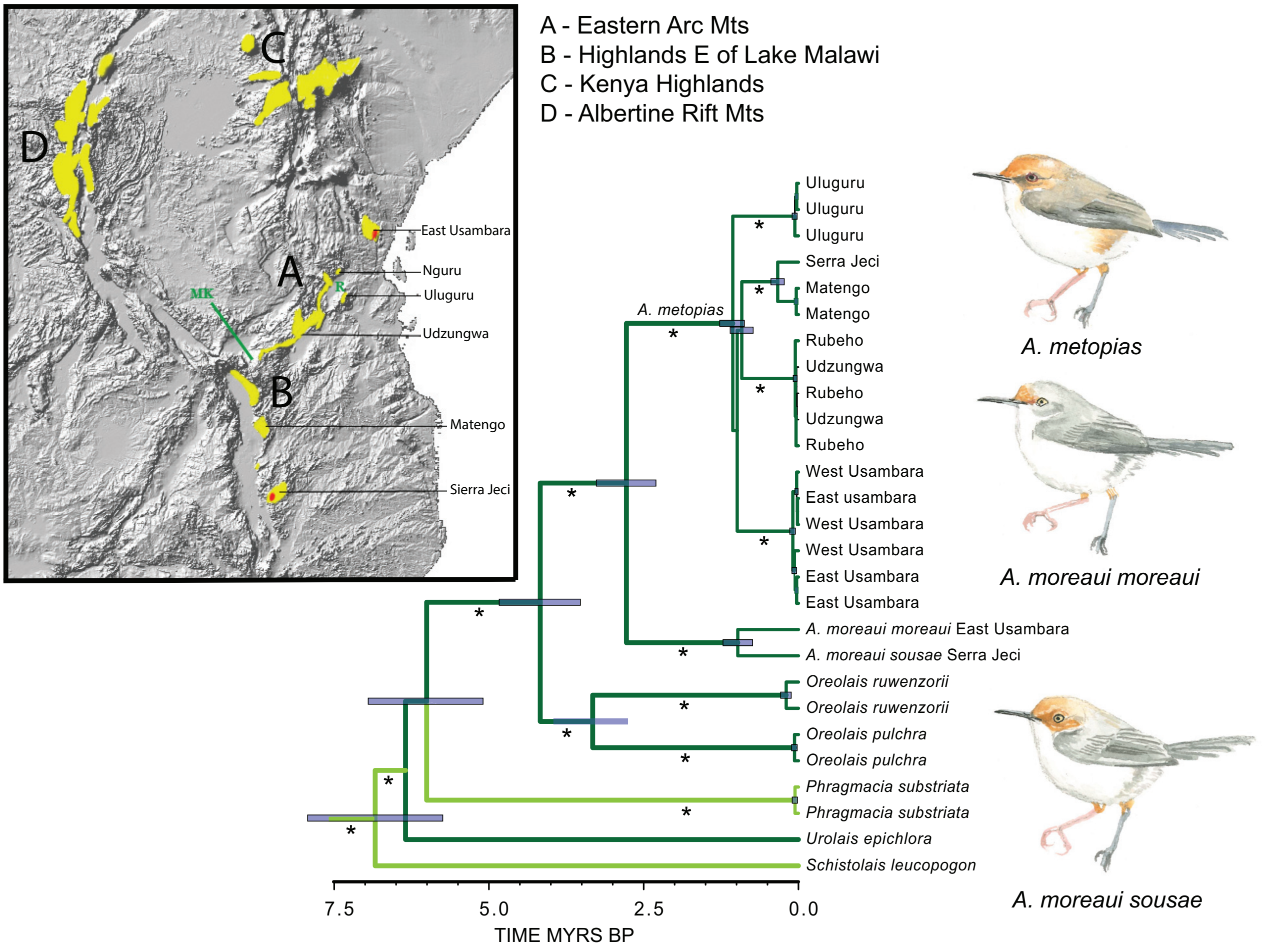

FIGURE 2 | Distributions of *Oreolais* species in the Albertine Rift (*O. ruwenzorii*) and Gregory Rift Mountains (*O. pulchra*) and *Artisornis* in the Eastern Arc Mountains (*A. metopias*, and the relict distribution of *A. moreaui* in red); and phylogeny of these taxa and the deeply divergent *Phragmacia* of South African arid lowland habitats, and *Urolais* and *Schistolais* of the Guineo-Congolian rainforest region. The phylogeny is derived from a maximum likelihood analysis of seven genes using 13 partitions detailed in Bowie et al. (2018). Timing of divergence was estimated using the rates of nucleotide substitution reported by Lerner et al. (2011). On the map: R, Rubeho Mountains; MK, the Makambako Gap delineating the southern extent of the Eastern Arc Mountains. * > 75% bootstrap support. In this phylogeny (and for following illustrations), lineages associated with montane forest are marked with dark green and those in lowland habitats with light green.

could have been more widely distributed through the eastern coastal lowland, but through climate change and anthropogenic modification of the landscapes, the species went extinct in most of the area, leaving behind the disjunct distribution we observe today.

Cisticolidae: The Plain-Backed Duetting Cisticolas

Within the largest genus of songbirds, the cisticola warblers (genus *Cisticola*), a small group of species with unstreaked dorsal plumage and with duetting songs, have long been recognized as a distinct montane clade with three species (Lynes, 1930; Davies, 2014): Chubb's Cisticola *C. chubbi* in the Cameroon Mountains and along the Albertine Rift to western Kenya; Hunter's Cisticola *C. hunteri* in the highlands of Kenya and northern Tanzania; and Black-lored Cisticola *C. nigriloris* in the Rubeho and Udzungwa highlands of Tanzania and the highlands flanking the northern end of Lake Malawi and adjacent Zambia (at 1,100–2,550 m). These Cisticolas inhabit montane forest, scrub, bracken and tall grassy vegetation associated with swamps and seeps (Urban et al., 1997; Ryan, 2006). A fourth member of this clade, the Kilombero Cisticola *C. bakerorum*, was recently described from the lowland reed-marshes of the Kilombero floodplain in southern Tanzania, a distinct ecological zone at only 240–305 m a.s.l from that occupied by the other three species in the clade (Fjeldså et al., 2021). The Kilombero Cisticola is sister to the geographically neighboring *C. nigriloris*, and molecular dating methods suggested that these two taxa diverged at the Pliocene-Pleistocene transition (2–3 Mya). Given the montane ancestral state reconstruction for the African duetting Cisticolas, a plausible evolutionary scenario is a down-slope dispersal of the ancestral Kilombero Cisticola that eventually led to the establishment and isolation on an ever-humid floodplain represented today by the lowland expanse of the Kilombero.

Platysteiridae: The Batis Flycatchers

Within the speciose genus *Batis*, the sexually dimorphic *Batis capensis* superspecies (Hall and Moreau, 1970) is primarily associated with montane forests and thickets that extend from northern Tanzania, through Malawi, the highlands of northern Mozambique and eastern Zimbabwe to reach the Cape Province in South Africa, where montane forest reaches sea level as latitude compensates for altitude. Traditionally considered a single species with: *mixta* occupying the costal forests of southeastern Kenya and the mountains of Tanzania and extreme northern Malawi; *sola* the mountain blocks east of Lake Malawi; *dimorpha* the mountains of southern Malawi and northern Mozambique; and several closely related taxa occupying the montane (*erythrophthalma*) and lowland hill habitats (*kennedyi*) of Zimbabwe and South Africa (*capensis* and *hollidayi*). An outlying lowland taxon, *reichenowi*, inhabits the lowland forests on the Rondo Plateau and other sites in the southeastern corner of Tanzania near the Mozambique border. Most of the populations from northern Malawi through Tanzania inhabit montane forest, although there is evidence of seasonal visits to wooded foothills (down to 540 m) in the dry season (Fjeldså et al., 2006, 2010), and some populations of *mixta* in the coastal zone of Kenya and northern Tanzania are resident to lowland forest like *reichenowi*.

A phylogeographic study by Fjeldså et al. (2006) demonstrated that the East Africa taxa (*mixta*, *dimorpha*, and *sola*) do not form a mophophyletic clade, because the Rwenzori Batis *B. diops* of the Albertine Rift Mountains is more closely related to *mixta* populations in coastal Kenya and the northern Tanzanian Mountains (extending to the Nguru Mts), whereas the populations inhabiting the highlands of south-western Tanzania are genetically distinct. Fjeldså et al. (2006) recognized the latter populations as a new species, the Dark Batis, *B. crypta*, which extends from north and east of Lake Malawi through the southern Eastern Arc Mountains to the Ukaguru Mountains in central Tanzania. Species rank for *B. crypta* was awarded because of reciprocal monophyly, diagnostic morphological characters, and the lack of evidence for gene flow with the Forest Batis *B. mixta*, although the two species are separated by only 25 km of hills (potential habitat in the non-breeding season) between the Ukaguru and Nguru Mountains. Intriguingly the phenotypically distinct *reichenowi*, which is isolated in the coastal forests of south-eastern Tanzania, was nested within *B. mixta* in the molecular phylogeny of Fjeldså et al. (2006) – a result corroborated by our expanded analyses in this paper (**Figure 3**), with the caveat that due to the lack of tissue samples, we are unable to determine the phylogenetic position of Woodward's Batis *B. fratrum*, which somewhat resembles *mixta* and *reichenowi* and inhabits the lowlands of Mozambique and southern Malawi.

In our expanded phylogenetic analysis that now includes populations of *Batis* distributed across the Malawi Rift and southern Africa (**Figure 3**), montane populations of the northern *sola* and southern *dimorpha* were recovered as monophyletic clades sister to the Cape Batis *B. capensis* of southern Africa. Similar to the parapatric distribution of *B. mixta* and *B. crypta* in the Eastern Arc Mountains, the ranges of *B. crypta* and *B. (capensis) sola* are separated by only 10 km of mid-altitude rangeland and palm savanna. The Albertine Rift *B. diops* is recovered as closely related to the *B. mixta/crypta/reichenowi* species complex; an interesting result given that *B. diops* is phenotypically monomorphic compared to all other populations in this study, which are dimorphic.

The geographically isolated population of *B. mixta reichenowi* (Fjeldså et al., 2006, treated as an independent species by del Hoyo and Collar, 2016) would represent a phylogenetic species (Cracraft, 1983) based on the distinctive appearance of the female plumage (**Figure 3**). The nested position of *reichenowi* in the phylogeny suggests that this small satellite population of *Batis* diverged by budding from the more widely distributed northern *B. mixta*, and is likely a relict population left after a humid period when coastal forest extended along the east coast of Africa, where genetic drift and rapid fixation of alleles (possibly the MC1R gene affecting expression of melanin; see Harris et al., 2020) resulted in the distinct plumage of *reichenowi*. The contraction of coastal forest as Africa became more arid through the Pleistocene and associated anthropogenic change likely left *reichenowi* as a relictual taxon now restricted to some lowland forests in south-eastern Tanzania.

Muscicapidae, Subfamily Cossyphinae: The African Robins

The African robins comprise a monophyletic clade of c. 45 insectivorous species (Fjeldså et al., 2020), with the majority of these species distributed in forest understorey and thickets across sub-Saharan Africa, especially in the lower montane zone (Voelker et al., 2010). The family as a whole, like many other families of "higher songbirds" (Passerida, see Fjeldså et al., 2020), shows a high thermal flexibility and tendency to radiate in montane regions. However, some species of robin-chats (*Cossypha, Dessonornis,* and *Caffrornis*) can be found in thickets at all elevations from the lowlands to the treeline, with a few species even penetrating arid savanna. To illustrate the elevational flexibility of these lineages we provide new analyses of two lineages [*Sheppardia* species (akalats), forest-chats in the genus *Chamaetylas*], and place these results in context with those from a published study of a third lineage, the White-stared Robin *Pogonocichla stellata* (Bowie et al., 2006).

The genus *Sheppardia* includes at least 10 species that form a monophyletic clade within the larger African forest robin assemblage (Voelker et al., 2010; Fjeldså et al., 2020). Akalat species are typically restricted to montane or lowland forest, with a few species (e.g., East Coast Akalat *S. gunningi*) occupying the entire elevational gradient. The greatest diversity of *Sheppardia* species occurs within the Eastern Arc Mountains of Tanzania (five species). These five species comprise three clades (**Figure 4**, see also Voelker et al., 2010). (1) The Usambara Akalat species complex *S. lowei-aurantiithorax-montana* primarily occupies the interior of well matured forest with dense shrubbery and liana tangles on upland plateaus at 1,400–2,400 m, with species replacing each other across the different montane highlands of the Eastern Arc Mountains (Beresford et al., 2004). (2) Sharpe's Akalat *S. sharpei* inhabits forests in the mid-montane zone

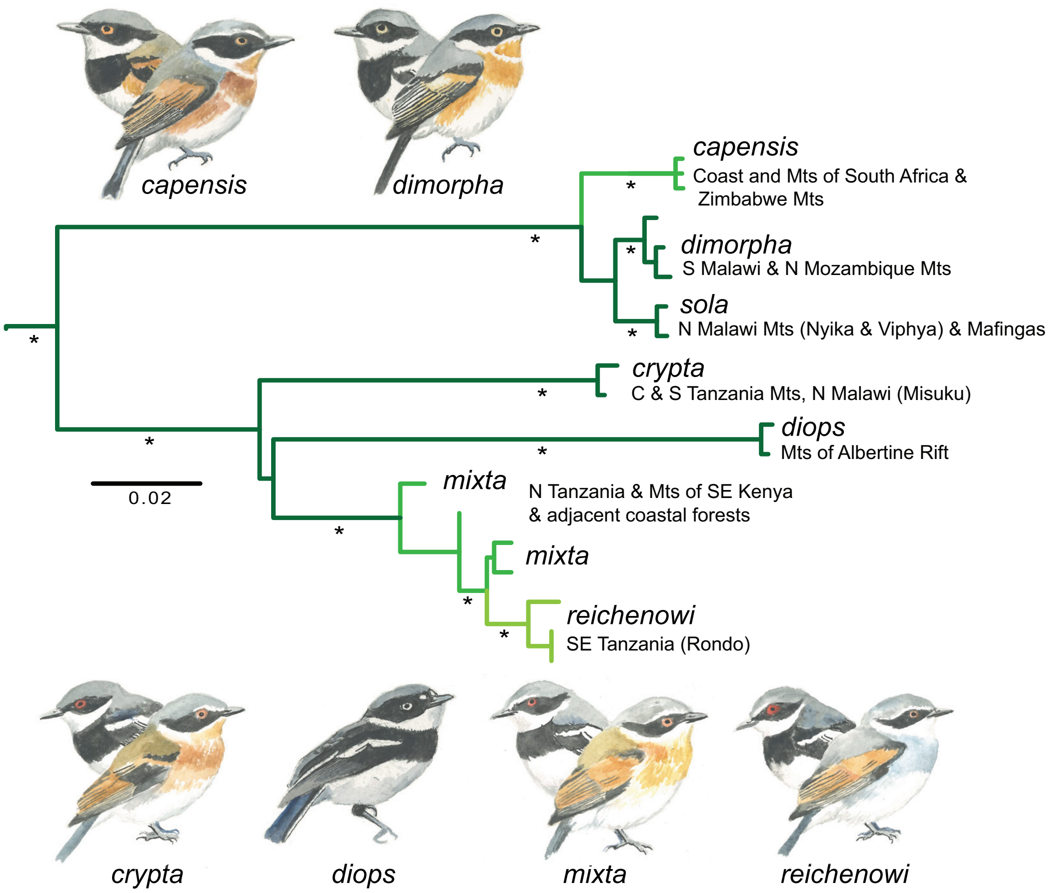

FIGURE 3 | Multilocus DNA (3 loci) phylogeny of *Batis* species of east and southern Africa derived from a partitioned maximum likelihood analysis. Dark green branches are indicative of lineages that occupy montane forest, light green is indicative of lowland forest, and the intermediate green color is indicative of lineages that occupy a broad elevation range extending into both lowland and montane forest. * > 75% bootstrap support.

(1,030–2,160 m) extending through most of the Eastern Arc Mountains, with subspecies *usambarae* occupying the northern Eastern Arc to the Nguru Mountains, and *sharpei* the remainder of the Eastern Arc, the volcanic highlands of southern Tanzania and the northern and central highlands of the Malawi Rift. Our present analysis (**Figure 4**) and that of Voelker et al. (2010) recover Sharpe's Akalat as sister to a clade comprising the lowland Gray-Winged Akalat *S. polioptera* and the elevationally variable Bocage's Akalat *S. bocagei*, although with relatively weak bootstrap support. (3) *S. gunning* is mainly found in lowland forest (to 300 m) along Africa's east coast, with the pale plumaged nominate subspecies *gunningi* occupying the coastal forests of north-central Mozambique. The subspecies *sokokensis* occupies the coastal forests of south-eastern Kenya and northern Tanzania, extending to Zanzibar Island. In contrast, the remaining two subspecies, which are more richly pigmented (**Figure 4**), are found in the lower montane zone; *bensoni* in northern-central Malawi and on Mount Mabu in northern Mozambique (500–1,750 m), and *alticola* on the steep Nguu Mountains (alias Nguru North) in Tanzania (850–1,750 m, Fjeldså et al., 2010). *S. gunningi* and *S. sharpei* do not overlap geographically: this results in the northern *alticola* and southern *bensoni* having disjunct ranges >600 km apart, and hence, it is surprising that they form a monophyletic clade (**Figure 4**) to the exclusion of more geographically proximate taxa (*sokokensis* and *gunningi*, respectively). This result lends further support to a past long-distance connection between northern Tanzania and northern Mozambique, consistent with interpretation of phylogeographic structure in the *Artisornis* warblers as described above (see also Bowie et al., 2018).

The White-Starred Robin *Pogonocichla stellata* is one of the most widespread Afromontane birds, occupying highlands around the montane circle of Africa, with its range extending through the Malawi Rift to the Western Cape in South Africa. Periods of aridity during the early- to mid-Pleistocene resulted in regional structuring of populations with breaks in gene flow separating populations in: (1) the Albertine Rift (ssp. *ruwenzorii*); (2) Kenyan Highlands (*keniensis*); (3) the northern Eastern Arc (*helleri*); and (4) central Eastern Arc and Malawi Rift mountains (Nguru to northern Mozambique; ssp. *orientalis*) (Bowie et al., 2006). Some sharing of mitochondrial DNA haplotypes occurs between *ruwenzorii* and *orientalis*, and between *helleri* and

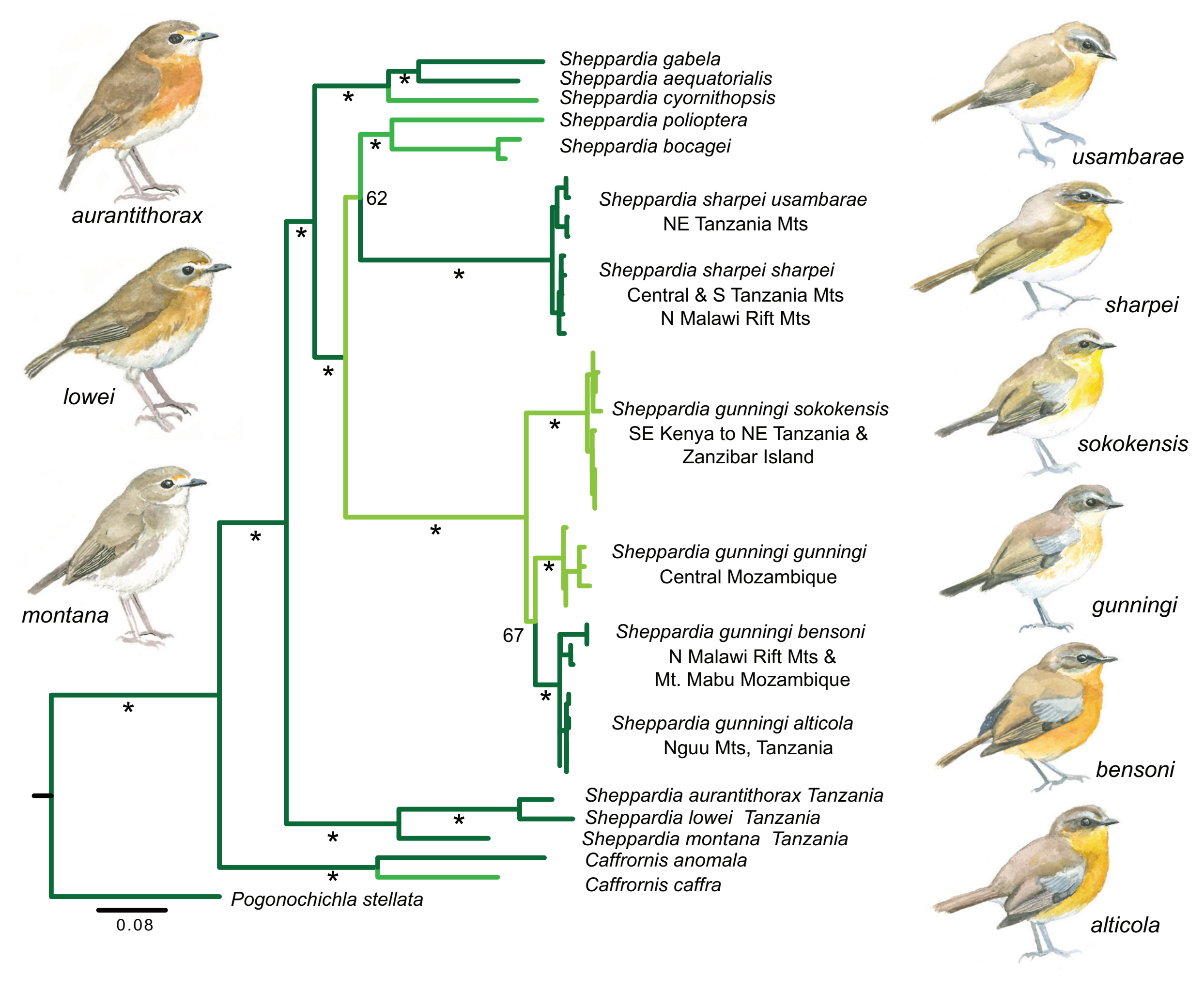

FIGURE 4 | Mitochondrial DNA (ATP6, ND2) phylogeny of *Sheppardia* akalats of Africa derived from a partitioned maximum likelihood analysis. Dark green branches are indicative of lineages that occupy montane forest, light green is indicative of lowland forest, and the intermediate green color is indicative of lineages that occupy a broad elevation range extending into both lowland and montane forest. * > 75% bootstrap support.

orientalis, with coalescent modeling suggesting that the sharing of haplotypes by *ruwenzorii* and *orientalis* is due to ancestral polymorphism rather than recurrent gene flow (Bowie et al., 2006). In contrast, gene flow seems to be taking place between the montane populations of northern Tanzania (*helleri*) and southern Malawi (*orientalis*) to the exclusion of the interior Eastern Arc Mountains and the central and northern Malawi Rift. It is likely that the White-starred Robin undergoes seasonal elevational migration with the coastal forests of Tanzania and northern Mozambique providing a "corridor" of connectivity, in a similar manner as has been inferred for the Long-billed Tailorbird and East Coast Akalat mentioned in the preceding case studies.

The chunky forest chats in the genus *Chamaetylas* inhabit the dark forest understorey, often foraging near swarms of driver ants (*Dorylus*), but in the dry season they are also found in riparian forests in the adjacent foothills (Fjeldså et al., 2010). Surprisingly, small/localized breeding populations, which have been classified as *C. fuelleborni xuthura* (Clancey and Lawson, 1969) have been found much further south, in lowland sand forests between Beira and the Zambezi River in Mozambique. Records of *Chamaetylas* forest chats from a coastal forest in southeastern Tanzania could be seasonal migrants from the Eastern Arc Mountains, but they could also represent a resident population and an outlier from the Mozambique lowland population (Jensen et al., 2005). Due to the presence of landmines, many parts of northern Mozambique remain difficult to explore, hence, the forest patches that occupy the hills and inselbergs of northern Mozambique are largely unknown.

The distribution of the White-chested Alethe *Chamaetylas fuelleborni,* with nominate *fuelleborni* occurring in the montane highlands of the Eastern Arc Mountains and the mountains flanking the northern Malawi Rift, and the form *xuthura* of the lowland forests in Mozambique, point to a past connection through the once more extensive coastal forests of Tanzania and Mozambique, probably reaching the Lebombo Mountains south of Maputo. We presently lack molecular data for *xuthura*, but should this taxon be sister to the montane Thyolo Alethe *C. choloensis* that occupies the mountains of southern Malawi, instead of *C. fuelleborni*, this would still illustrate extraordinary elevational flexibility in these forest chats.

Pellorneidae, the Jungle Babblers

The genus *Illadopsis* comprises eight insectivorous species mainly inhabiting understorey habitats in the Guineo-Congolian rainforests. However, Pale-breasted Illadospis *Illadopsis rufipennis* has a sister species, Mountain Illadopsis *I. pyrrhoptera*, in the montane forest of the Albertine Rift and an isolated population in northern Malawi. Small local populations of Illadopsis in Tanzania have traditionally been referred to as subspecies (*distans* or *puguensis*) of *I. rufipennis*), Molecular phylogenetic analysis places the Tanzanian populations as a sister-group to the montane *pyrrhoptera*, and the West African *I. rufipennis* populations as sister to this clade. Hence, the traditional *I. rufipennis* was not monophyletic (see also Nguembock et al., 2009) and the Tanzanian populations are now recognized as a separate species *I. distans* (del Hoyo and Collar, 2016). Further, a high degree of phylogeographic structure is recovered among the Tanzanian populations sampled (**Figure 5**), with deep genetic divergence (5–7% uncorrected) suggesting that these populations started to diverge over the past 2 Mya. These populations occupy both lowland and montane forest, and are subtle morphologically different.

One Tanzanian lineage (nominate *distans*) is found in montane forest in the Usambara Mountains and further inland in foothills of the Nguru Mountains and adjacent Mount Kanga. Another lineage (still unnamed) is distributed locally in the Rubeho and Udzungwa Mountains, mainly occurring in shady places along forest streams up to 2,000 m, but locally (or seasonally) also in adjacent foothill forests (Fjeldså et al., 2010). A third lineage (spp. *puguensis*) is found in some lowland forests near Dar es Salaam and further south near the Rufiji Delta, and genetically similar birds are found in groundwater forests in the foothills of the Uluguru Mountains 160 km inland, and (surprisingly) in montane forest in the Kiboriani Mountains. A small lowland population on Zanzibar Island has not yet been included in molecular phylogenetic analysis. These populations are highly fragmented and are of critical conservation concern.

Pycnonotidae, Greenbuls of the Genus *Phyllastrephus*

This group, with at least 18 insectivorous species, is mainly distributed in lowland gallery forests with dense vine-tangles and epiphytes. Adaptation to higher elevation occurs among members of the Yellow-streaked Greenbul *Phyllastrephus flavostriatus* complex, in Cabanis's Greenbul *P. cabanisi* and Placid Greenbul *P. placidus*, and in two local populations of the Tiny Greenbul (see below). Further, the Gray-olive Greenbul *P. cerviniventris* of central and eastern Africa is mostly associated with riparian and groundwater forest in montane foothills, but it is also found locally in small riparian thickets or forest swamps up to 1,900 m in Malawi and Tanzania (e.g., Dowsett-Lemaire and Dowsett, 2006; Fjeldså et al., 2010; John and Kiwango, 2021).

Phyllastrephus debilis, which has an isolated position in the phylogeny of the genus (Johansson et al., 2007; Fjeldså et al., 2020), is found across the mosaic of coastal forests in eastern Africa, with subspecies *debilis* occurring in central Mozambique, and subspecies *rabai* through the coastal zone of Tanzania and southern Kenya. Distinctly larger birds with more saturated plumage colors inhabit montane forests between 600 and 2,150 m in the West Usambara and Nguru Mountains of Tanzania (ssp. *albigula*). A multilocus study of 124 specimens from all parts of the range placed *debilis* and *rabai* close together but revealed that the *albigula* populations had been genetically isolated in their montane forest habitats since 2.4–3.1 Mya (Fuchs et al., 2011). The montane-lowland populations are now in secondary contact in Tanzania, as *rabai* has expanded inland and reaches the lower montane forest on the slopes of some Eastern Arc Mountains. This has resulted in limited recurrent gene flow from *rabai* to *albigula*, but the populations appear to have retained their integrity and are now recognized as separate species (Fuchs et al., 2011; Gill et al., 2021). The molecular data could not support or reject the possibility of ongoing gene flow between the montane populations of *albigula* on the Nguru and Usambara Mountains, which are presently isolated by 125 km of dry lowland plains.

Phyllastrephus flavostriatus is phenotypically and genetically complex. A phylogeographic study based on 248 specimens representing nearly every known allopatric population and using two mitochondrial markers (Lokugalappatti, 2011), placed populations in montane forests distributed across the Albertine Rift (subspecies *graueri*, *olivaceogriseus, itombwensis,* and *kungwensis*) as the sister clade to populations inhabiting variable elevations in southern and eastern Africa; we recover similar results in our analyses (**Figure 6**). A distinct morphological form (ssp, *alfredi*, with a brown instead of gray head) inhabits highlands along the Rukwa and Malawi Rifts and may be the first diverging lineage within the Yellow-streaked Greenbul complex, or may form a clade sister to a complex of populations in the Udzungwa Mountains (*uzungwensis*) and mountain ranges and foothills extending from southern Malawi to the KwaZulu-Natal Province of South Africa (ssp. *vincenti* and *flavostriatus*; Lokugalappatti, 2011). Nested among these southern populations are populations (spp. *tenuirostris*) that inhabit coastal forests from northern Mozambique to southern Kenya, with individuals ascending to montane forest in the northern Eastern Arc Mountains. These data suggests an origin in montane habitats and development of a broad ecological niche during a Pleistocene expansion toward southern Africa, with a final dispersal through the coastal forest zone of East Africa to the adjacent montane forests of the northern Eastern Arc Mountains.

Lybiidae, African Barbets

This family of fruit-dependent birds is widespread in African lowland forest and woodlands. The Green Barbet *Stactolaema olivacea*, with three widely disjunct subspecies primarily restricted to lowland forest (*olivacea*, *woodwardi,* and *hylophona*; **Figure 7**) and three subspecies restricted to montane forest

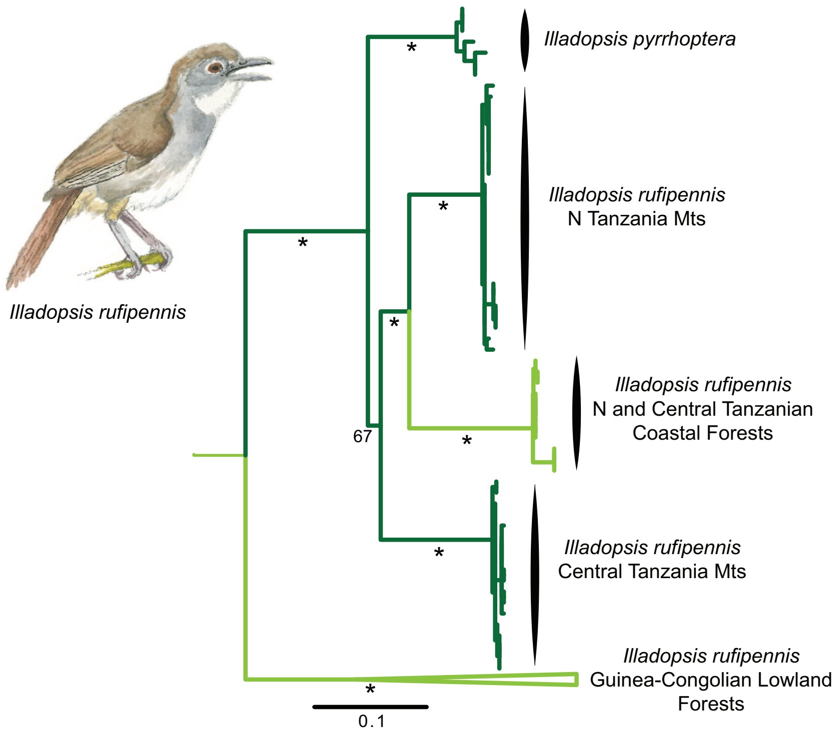

FIGURE 5 | Mitochondrial DNA (ATP6, ND2) phylogeny of *Illadopsis* species of east Africa derived from a partitioned maximum likelihood analysis. Note that I. *pyrrhoptera* renders *I. rufipennis* polyphyletic. Dark green branches are indicative of lineages that occupy montane forest, intermediate green is indicative of lineages that occupy a broad range of elevations and light green lineage occupies lowland forest. * > 75% bootstrap support.

(*belcheri*, *rungweensis,* and *howelli*), provides an ideal exemplar taxon with which to explore speciation patterns among lowland and montane forests, as well as the age and extent of connectivity between these habitats. Particularly intriguing is Woodward's Barbet (*C. olivacea woodwardi*), that occurs only in the Ongoye (Ngoye) Forest in KwaZulu-Natal, South Africa, over 2,000 km from its nearest neighboring population in southern Malawi (*belcheri*). Due to the sharing of bright yellow-green ear coverts, the taxa *woodwardi* and *hylophona* from coastal forests in south-eastern Tanzania (Jensen et al., 2005), are traditionally placed as sister taxa (Clancey, 1989) and are often lumped together as the same species (Sinclair and Ryan, 2010).

Our molecular analyses reveal remarkably shallow sequence divergence among all six subspecies of Green Barbet (max. 2.36%) despite the very large distances separating disjunct populations (**Figure 7**). This shallow sequence divergence makes the relationship among subspecies difficult to resolve. Three clades are recovered in our molecular phylogenetic analyses. (1) A clade of individuals sampled from the lowland Sokoke Forest along the coast in southeastern Kenya and individuals sampled from the montane forests of the East and West Usambara Mountains (subspecies *olivacea*). (2) A clade of individuals occupying the montane highlands of the remainder of the Eastern Arc Mountains and the Misuku Hills in extreme northern Malawi (subspecies *howelli* and *rungweensis*). (3) A clade comprising three widely disjunct taxa, with *woodwardi* (lowland) and *belcheri* (montane) recovered as sister-taxa, and *hylophona* (lowland) putatively sister to these two taxa. These data point to at least two instances of montane to lowland transitions, suggesting a recent history of dispersal between lowland and montane habitats for this canopy feeding bird.

Additional Putative Cases of Montane-Lowland Range Dynamics in East African Birds

In order to underscore that flexibility in elevational distribution is not just a rare anomaly, we mention below some further cases, which have not yet been adequately evaluated by phylogeographic methods. The many cases of montane species that are nested within larger clades of lowland bird species, provide evidence of past flexibility in elevational distribution.

Superfamily Sylvioidea, "Warblers" in the Broader Sense

Colorful species in the genus *Apalis* are mainly distributed in canopies of montane forest areas in Central Africa (*A. personata*, *binotata* and *jacksoni*) and the outlier highlands of Cameroon and Angola (*A. binotata* and *jacksoni*), with one distinctive species, the White-winged Apalis *A. chariessa*, with a relictual distribution in East Africa. One population of White-winged Apalis is found in mid-elevation rainforest in southern Malawi (Dowsett-Lemaire and Dowsett, 2006), another within the humid montane forests of the Udzungwa and Uluguru Mountains, and

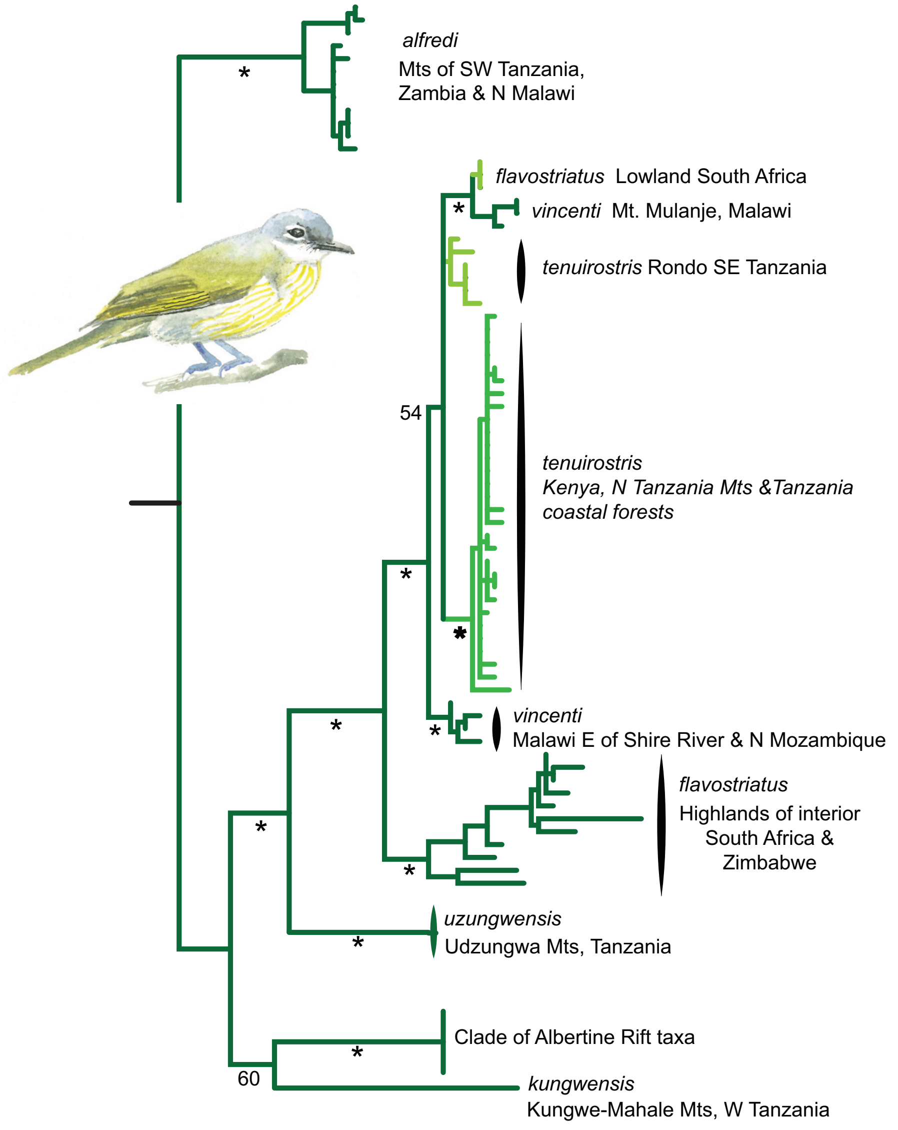

FIGURE 6 | Mitochondrial DNA (ND2) phylogeny of Yellow-streaked Greenbul *Phyllastrephus flavostriatus* populations across east and southern Africa derived from a partitioned maximum likelihood analysis. Dark green branches are indicative of lineages that occupy montane forest, light green is indicative of lowland forest, and the intermediate green color is indicative of lineages that occupy a broad elevation range extending into both lowland and montane forest. * > 75% bootstrap support.

the species also once occurred (at least until 1961) 570 km further north, in the lowland forests of the lower Tana River delta near the Kenya coast. Another interesting case is the Black-headed Apalis *Apalis melanocephala*, which is patchily distributed from northern Mozambique and from Malawi to Kenya, mainly in highlands but locally also in foothills and coastal forests. Examining material in several museum collections, JF found significant variation in plumage melanization and tail length, both within and between local populations. Age-related variation and polymorphism could play a role as it does in Cisticolas, but there are also signs of introgression between populations in the south (subspecies *tenebricosa*) and between populations in Tanzanian coastal forests or mountains in the northern part of the Eastern Arc Mountains (ssp. *moschi*).

Grass warblers of the genus *Bradypterus* are patchily distributed across sub-Saharan Africa, mainly occurring within the humid undergrowth of montane forests, or in swamp habitats (Kahindo et al., 2017). The Little Rush-warbler, *B. baboecala*, is genetically structured with local populations both in highlands and lowlands in central and eastern Africa (unpubl. data).

White-eyes, genus *Zosterops,* represent one of the most remarkable avian cases of rapid radiation in the Pleistocene. Although most of the diversity is found in the Indo-Pacific archipelago, one lineage colonized Africa and diversified throughout the sub-Saharan continent (Cai et al., 2019; Gwee et al., 2020). Recent molecular work suggest that the many populations, which are characterized by broad, white eye-rings and replace each other in different Afromontane areas,

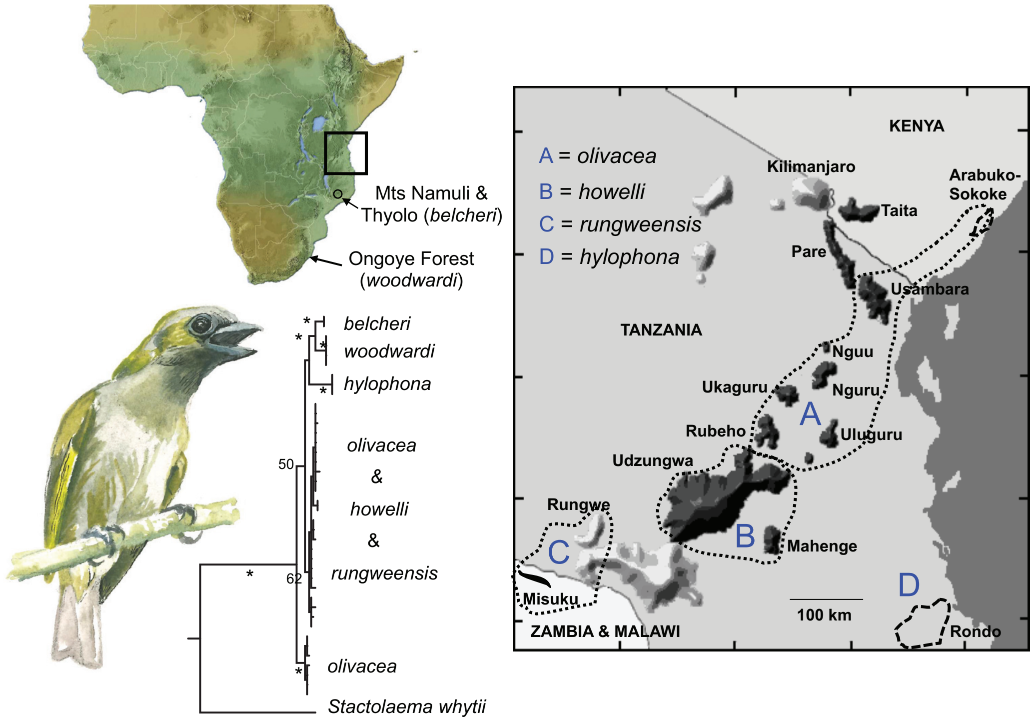

FIGURE 7 | Top Left Panel: Distribution map of the Green Barbet *Stactolaema olivacea*. Right Panel: An enlargement of the box demarcated on the map of Africa depicting the distribution of Green Barbet subspecies across Tanzania and extreme northern Malawi. Bottom Left: Mitochondrial DNA (Cytb, ND3) phylogeny of Green Barbet populations across east and southern Africa derived from a partitioned maximum likelihood analysis. * > 75% bootstrap support.

do not represent a monophyletic clade but are interspersed in the phylogeny among generally more widespread lowland forms (Martins et al., 2020). This suggests that numerous local populations need to be recognized as independent species (Pearson and Turner, 2017). The results could also be interpreted to suggest several independent cases of speciation along local elevational gradients (Cox et al., 2014), but, given uncertainties about the phylogenetic resolution and remaining gaps in the geographical sampling, it is difficult to exclude the alternative interpretation, that this "island speciator" diversified rapidly across Africa's "sky archipelago," to subsequently disperse and settle in the surrounding lowlands.

Nectarinidae, Sunbirds

The sunbirds of Africa have mainly radiated in upland savannas and mountain regions (Bowie, 2003). Recent phylogenetic work has revealed a monophyletic group, *Euchloridia* (Bowie and Fjeldså, 2020), which is highly heterogeneous in plumage characters, bill shape and diet, resulting in its constituent species having previously been placed in different genera, which we refer to below to avoid confusion. This group appear to represent relict populations from the time when the Miocene evergreen forests extended across tropical Africa from west to east. Most constituent species are dull olive-green birds of lowland rainforest (*Deleornis* and *Anthreptes*), but the Banded Green Sunbird *Anthreptes rubritorques* is a rare inhabitant of mid-elevation forest on some Eastern Arc Mountains. The more colorful East African species Plain-backed Sunbird *Anthreptes reichenowi* occupies coastal forests, the phenotypically aberrant Rufous-winged Sunbird *Cinnyris rufipennis* is restricted to wet highland forest in the Udzungwa Mountains and its sister-species, the Amani Sunbird *Hedydipna pallidigaster*, has a peculiar, patchy distribution, with a small population in the Udzungwa highland forest, another in the East Usambara Mountains, and yet another in coastal forests in Kenya, mainly in Arabuko Sokoke Forest (Fjeldså et al., 2010). These three populations appear to be genetically closely related (unpubl. data) but inhabit very different climates. Thus, even within this relatively old clade with deep divergence between constituent species, we see mixed occupancy of lowland and montane habitats, and elevational flexibility among populations within one species.

The Olive Sunbird *Cyanomitra olivacea* (now *Haagneria*; Bowie and Fjeldså, 2020) one of Africa's most widespread songbirds occupies both montane and lowland forest throughout its range. Analyses by Bowie et al. (2004) of mitochondrial DNA sequence variation across the species range revealed that that birds sampled from the northern Eastern Arc (Taita Hills, Pare, Usambara, and Nguru Mountains) share alleles from the same haplotype clusters as birds from southern Malawi (Mt. Zomba) and northern Mozambique (Mt. Namuli) to the exclusion of the interior Eastern Arc Mountains (Uluguru, Rubeho, and Udzungwa). This suggests that lowland coastal forests along Africa's east coast have served as a corridor linking coastal forests in the north with those in extreme southeastern Tanzania, and Mozambique, in a similar manner as has been inferred in several of the case studies mentioned above.

Other potential cases of widespread montane forest birds with local populations in coastal forests comprise Eastern Bronze-naped Pigeon *Columba delegorguei*, Lemon Dove *Columba larvata*, Silvery-cheeked Hornbill *Bycanistes brevis* and Black-fronted Bush-shrike *Chlorophoneus nigrifrons*. The green turacos, the *Tauraco persa* group, present a very complex case with diverse distributions, occupying both montane and lowland habitats, and would present a very interesting case for detailed phylogeographic study.

A Concise Summary of Our Findings

Our data analyses reinforce earlier views (e.g., Moreau, 1966) that the distribution of forest birds across East Africa is complex. However, through the synthesis of the above case studies we are able to identify some repeated patterns. First, there are some bird species with populations in the Eastern Arc Mountains and in adjacent lowland forests or in the northern coastal forests (*Cisticola*, *Illadopsis*, *Phyllastrephus albigula/debilis*, *Zosterops*, and *Hedydipna pallidigaster*). Second, several species distributed in the branch of the Eastern Afromontane Biodiversity Hotspot located in Malawi, or in the lowlands of Mozambique, also have resident populations in the northern coastal forests or in adjacent mountains in the northern Eastern Arc to the exclusion of the central Eastern Arc Mountains (*Artisornis moreaui*, *Batis* spp., *Sheppardia gunningi*, *Phyllastrephus flavostriatus*, *Stactolaema olivacea*, and *Apalis* spp.), and there are indications of gene-flow between these areas (*Pogonocichla stellata*, *Cyanomitra olivacea*). Third, some of the populations appear to be relictual, with several montane species nested within clades of mainly lowland species (see Fjeldså et al., 2020 for comprehensive species phylogenies) and *vice versa* (e.g., *Batis* spp.). If we take into account also the many bird species with mixed elevational distributions, which have not yet been included in detailed phylogeographic studies, we judge that approximately 15% of all forest-dependent and forest-associated species of East Africa have mixed elevational distributions presently.

Flexible elevational distributions are mainly seen among birds of the forest understorey or mid-canopy, and most of the species are insectivorous. This is for instance the case with the *Phyllastrephus* greenbuls, while other greenbul genera, which have mixed (insect/fruit) diets, are mainly associated with lowland forest, or specialized to live in montane forest (*Arizelocichla*). White-eyes and sunbirds have mixed diets (insects and nectar) and *Stactolaema* is fruit-dependent.

DISCUSSION

The flexibility in elevational distributions of birds in East Africa is quite distinct from what is described for avifaunas of larger and more connected montane regions at low latitudes, such as the Sino-Himalayan Mountains (Päckert et al., 2012) or the South American Andes, where lineages tend to evolve through geographical isolation within narrow elevational bands, with segregation into different ecological zones coming secondarily, by ecological segregation of independent and competing species (e.g., Cadena et al., 2012; Cadena and Cespedes, 2020; Linck et al., 2021).

The rather fuzzy African situation could possibly be interpreted as a consequence of the nature of African mountains as isolated enclaves, or "sky islands" (Bowie, 2003; Fjeldså and Bowie, 2008; Voelker et al., 2010). Most of the African mountains are only moderately high (<2,500 m), and given the instability of the African climate and high seasonality of the intervening savanna matrix habitats, the existence of habitats that would facilitate dispersal of montane birds across lowland habitats may have fluctuated considerably, on timescales from decades through millennia (Nicholson, 2000), and especially through the higher-amplitude Pleistocene glacial periods (deMenocal, 2004; Trauth et al., 2005). This instability, where suitable habitat may not have persisted through climate cycles in some montane highlands (Bowie, 2003), suggests that in order for many African forest birds lineages to survive, they had to exhibit considerable flexibility in their use of different ecological zones. Some species survive only as relict populations in those highlands where remnants of the ancient African rainforest environment had been maintained over evolutionary time (Voelker et al., 2010). Some birds may have been able to survive through flexible use of food resources, for instance by being able to shift their diets between fruits, grain and insects, or by using different microhabitats (interior forest vs. treefall gaps or edges). However, some of the specialized insectivores appear to be sensitive and must leave their breeding habitat during the dry season (Mulwa et al., 2012)[2]. Most of our cases of flexible elevational distributions are insectivores. Little is known about where birds move in the dry season, but casual observations in Tanzania of highland birds in riparian habitats in the montane foothills or in near-by patches of lowland forest suggest that most birds do not move far from their highland breeding habitat (Burgess and Mlingwa, 2000; Bowie et al., 2006), although still far enough to sometimes end up in the neighboring sky islands on their return migration.

Seasonal elevational migrations could lead to establishment of resident populations in lowland sites that are hydrologically stable, as is the case for *Cisticola bakerorum* highlighted above, and the case described for *Phyllastrephus debilis* suggests that lowland lineages can also adapt to occupy montane forest. When moving between cool highlands and hot lowlands, the birds will have to adapt to markedly different temperature regimes, although some groups may be thermally flexible (Khaliq et al., 2015) and other factors, such as soil humidity, amount of green foliage and local abundance of insects could be more important than temperature. The existence of breeding populations in Arabuko-Sokoke forest in coastal Kenya of some birds that otherwise inhabit montane forest could simply reflect the large extent of this forest, which allows it to harbor viable populations. The complex distributions of highland and lowland forms of, for instance, *Illadopsis* and *Zosterops*, suggests that shifts between

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[2] Dinesen, L., Lehmberg, T., Romdal, T. S., Sonne, J., and Hansen, L. A. (in review). Seasonal change in bird species community in the Udzungwa Mountains – an Afromontan evergreen forest in Tanzania. *Front. Ecol. Evol.*

different elevational climates may not be a significant challenge for these lineages. Based on the molecular data presented here, most of the elevational shifts took place during the Pliocene and Pleistocene, when Africa's climate was overall unstable, as documented from analyses of pollen in cores drilled from lake bottoms (Lyons et al., 2015), which indicate that rainfall regimes varied extensively across the continent (Trauth et al., 2005), with abnormally dry conditions during glacial periods (Lyons et al., 2015).

Above we presented several independent cases of connections between the mountains along the Malawi Rift or the adjacent Zambezian savanna region and the northern section of the coastal forests mosaic (including montane habitat islands in the northern part of the Eastern Arc Mountains), to the exclusion of the resident populations in the central and western Eastern Arc Mountains. This is manifest as indications of gene flow as well as cases of apparent long-distance vagrancy and establishment of highly disjunct distributions, as seen in *Artisornis moreaui* and *Sheppardia gunningi*. Given the number of such cases, this appears to be a repeated pattern, which could reflect one or more past connections across the lowland habitats of East Africa. Genetic indications of past range fragmentation in *Pogonocichla stellata* and *Cyanomitra olivacea* corresponds to the start of cooling of the northern biomes from the late Pliocene (Bowie et al., 2004, 2006).

We can assume two different scenarios for connectivity between the northern Eastern Arc and southern Malawi Rift. Some species may have been more widespread across forested landscapes in northern Mozambique and southern Tanzania, for instance in extensive bamboo forests or in patches of evergreen forest associated with inselbergs. As Africa became arid, lowland forests retreated and once-connected populations become fragmented, leaving remnant populations in the south and north (e.g., *Artisornis,* Bowie et al., 2018), and eventually also in south-eastern Tanzania [*Batis* (*mixta*) *reichenowi, Stactolaema olivacea hylophona*].

Another possibility is that birds breeding in the south (Malawi Rift, Mozambique, or Zambezian savanna region) migrated because of seasonal dryness, to reach "wintering" areas in the northern coastal forests. The coastal zone of northern Mozambique is in the rain shadow of Madagascar, and therefore does not receive the same predictable rainfall as the northern coastal forests and the montane forests of the Eastern Arc Mountains of Tanzania. The mountains of the Malawi Rift are influenced by local convectional rainfall cycles, which has been more variable over time than that of the Eastern Arc Mountains (Lovett, 1993). Conditions may therefore have been unstable over time, and in order to maintain breeding populations in the south, the forest birds would have to be ecologically flexible with vagrancy or seasonal migrations becoming an integral part of their life cycles (confer Winger et al., 2018). It is important in this context to note that the rainfall in the northern part of the coastal zone is concentrated around the equinoxes, with peak rainfall in April–May, and a weaker peak in October–November. This means that this zone is still lush and green, with abundant insect life, when Mozambique has its dry season, and this cycle of rain in the northern coastal zone is assumed to have been stable over evolutionary time (Prell et al., 1980; Marchant et al., 2007; Mumbi et al., 2008). This provides opportunities for birds breeding in the south to migrate north, where they may have been able to settle and breed in suitable places with a more predictable rainfall in the northern part of the Eastern Arc Mountains. The establishment of new populations through flexibility in migratory systems is analogous with recent (re)interpretations of other migratory systems, such as the rapid establishment of Nearctic migratory groups throughout the Neotropics (Winger et al., 2014) or the establishment of Sylvia warblers (of Palearctic origin) across Africa through migratory dropoff (Voelker et al., 2009; Voelker and Light, 2011).

In summary, we suggest that the extent of interactions between montane and lowland bird communities in East Africa has been underestimated. We present phylogenetic data that demonstrate how elevational flexibility has likely been selected for over evolutionary time and hypothesize that seasonal altitudinal migration between montane and lowland habitats is not only important at ecological time scales, but has likely played a role in facilitating the diversification of East Africa bird species. We urgently need to apply new methods of animal tracking East African forest bird communities in order to better understand how and when montane and lowland bird species move among habitat patches.

DATA AVAILABILITY STATEMENT

This study forms a synthesis of primarily previously published research, with updated analyses of these data. Molecular data are available on GenBank and were drawn from the following publications: Beresford et al. (2004), Bowie et al. (2004, 2006, 2018), Fjeldså et al. (2006, 2021), Nguembock et al. (2009), Voelker et al. (2010), Lokugalappatti (2011), with GenBank numbers provided in these publications.

ETHICS STATEMENT

This animal study was reviewed and approved by the University of California, Berkeley (IACUC R317, 2014-10-6780, 2016-04-8665).

AUTHOR CONTRIBUTIONS

JF made all paintings of birds used in the figures. Both authors contributed to the article and approved the submitted version.

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Patterns and Predictors of Small Mammal Phylogenetic and Functional Diversity in Contrasting Elevational Gradients in Kenya

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Mountains of the Afrotropics are global biodiversity hotspots and centers of speciation and endemism; however, very few studies have focused on the phylogenetic and functional dimensions of Afromontane small mammals. We investigated the patterns and mechanisms of small mammal phylogenetic and functional diversity and assembly along elevational gradients in Mount Kenya, the second highest mountain in Africa, and a contrasting low mountain range, Chyulu Hills. We sampled 24 200-m interval transects in both sites; 18 in Mt. Kenya (9 each in the windward side, Chogoria, and the leeward side, Sirimon) and 6 in Chyulu. We extracted the mitochondrial *Cytochrome b* gene to reconstruct a time-calibrated species tree for estimating phylogenetic diversity indices [phylogenetic richness (PD), mean nearest taxon distance (PD^{MNTD}), and nearest taxon index (PD^{NTI})]. A functional trait data set was compiled from the field-recorded measurements and published data sets for estimating functional diversity indices [functional richness (FD), mean nearest taxon distance (FD^{MNTD}), and nearest taxon index (FD^{NTI})]. Several environmental variables representing water-energy availability, primary habitat productivity, and topographic heterogeneity were used to estimate the predictive power of abiotic conditions on diversity variances using generalized linear and generalized additive regression models. The PD and FD peaked around mid-elevations in Mt. Kenya, unimodally increased or decreased in Chogoria and Sirimon, and monotonically increased in Chyulu. The divergence and community structure indices—PD^{MNTD}, FD^{MNTD}, and PD^{NTI} and FD^{NTI}—were relatively weakly associated with elevation. Overall, the tendency of assemblages to be phylogenetically and functionally closely related than expected by chance decreased with elevation in Mt. Kenya but increased in Chyulu. Across the indices, the annual precipitation and topographic ruggedness were the strongest predictors in Mt. Kenya, evapotranspiration and temperature seasonality were the strongest predictors in Chyulu, while temperature seasonality and terrain ruggedness overlapped as the strongest predictors in Chogoria and Sirimon in addition to annual precipitation in the latter and normalized difference vegetation index in the former. The observed contrasting trends in diversity distribution and the strongest predictors between elevational gradients are integral to the sustainable management of the high faunal biodiversity in tropical Afromontane ecosystems.

Keywords: Afromontane, biodiversity, Chyulu Hills, elevation gradient, Mount Kenya, small mammals, tropics

INTRODUCTION

Understanding species ecological and evolutionary spatial patterns and mechanisms underlying assembling patterns remains of heightened ecological interest in mountain ecosystems, where sharp variations in climate and space along elevational gradients dynamically structure communities over short distances (Rahbek et al., 2019a; Perrigo et al., 2020). In tropical mountains, rapid climate and vegetation turnover along elevational gradients maintain climatically stable conditions compared to surrounding areas, thus these mountains provide an ideal background for studying ecological and evolutionary processes that yield and preserve high faunal biodiversity of the tropics (Graham et al., 2014). Despite mountains of the Afrotropics being one of the most biodiverse regions globally, they are increasingly fragmented and isolated by expanding human activities (Raven et al., 2020; Rowan et al., 2020), which potentially restructure the ecological–evolutionary nexuses, especially for non-volant small mammals whose life histories and ecologies make them more vulnerable to such changes (Rowe and Terry, 2014). Moreover, while taxonomic diversity distribution patterns of mountains are considerably well studied in the Afrotropics, phylogenetic and functional dimensions have been of much less interest (McCain and Grytnes, 2010; Graham et al., 2014; Quintero and Jetz, 2018). There is a need for more studies on the Afrotropic mountains based on species evolutionary and ecosystem functioning dimensions to refine the integrated understanding of how environmental changes drive species assembling to enable effective biodiversity conservation actions.

Globally, mountain diversity-elevation associations are dominated by unimodal trends where the highest species richness occurs around the middle elevations (McCain and Grytnes, 2010; Guo et al., 2013; Quintero and Jetz, 2018). However, variations have emerged, where monotonic declines or increases have also been observed (McCain and Grytnes, 2010; Taylor et al., 2014; Camacho-Sanchez et al., 2019), suggesting that elevation-diversity associations may be more complex than studies have so far revealed, especially when local assembling drivers are considered. The phylogenetic and functional diversity distributions along elevational gradients in the tropics have largely been congruent with those of species richness, i.e., the highest phylogenetic and functional diversity at mid-elevations. For instance, Dreiss et al. (2015) observed that rodent assemblages from low-elevation forests in the Peruvian Andes were phylogenetically and functionally more diverse than the higher elevations, Montaño-Centellas et al. (2020) observed phylogenetic and functional overdispersion of birds in upper elevations, while He et al. (2018) and Zhang et al. (2020) observed that the functional and phylogenetic diversity of passerine birds in low elevations was higher than by expectation—overdispersed. Studies on how environmental variables influence species assembling (clustering or overdispersion) along elevational gradients continue to shed light on the intricate ecological and evolutionary processes in tropical mountain biodiversification, but with very few of them dedicated to the Afrotropical mountains. There is a need for more studies of poorly documented Afromontane mountains to disentangle the phylogenetic and functional diversity trends along elevational gradients, which can then inform more ecologically informed biodiversity management actions (Brum et al., 2017).

Mammalian elevation-diversity patterns in the tropics are mainly structured by climate, habitat productivity, and topography (Hawkins et al., 2003; Brown, 2014). For instance, the climate stability hypothesis has been widely explored to explicate why tropical habitats with less seasonal climates (precipitation and temperature) tend to have higher species richness compared to habitats with more seasonal climate regimes (Davies et al., 2009). Similarly, the productivity hypothesis relates to positive associations of species richness with increasing habitat productivity, which is implicated in positive correlations between resource availability and niche opportunities (Dreiss et al., 2015; McCain et al., 2018; Ramirez-Bautista and Williams, 2019). Notably, these hypotheses are mostly associated with global elevation-diversity patterns, meaning that local montane communities may be structured by systematically contrasting abiotic variables (Montaño-Centellas et al., 2020).

In this study, we investigated the patterns of elevational distribution of multiple phylogenetic and functional richness, divergence, and community structure dimensions using a comprehensive elevational stratified sampling of small mammals in Mount Kenya and the Chyulu Hills. We also analyzed the predictive influence of several environmental variables on the diversity distribution trends. We simultaneously used the phylogenetic and functional diversity due to the non-exclusive nature of the assumptions of evolutionary history and ecosystem functions (Cavender-Bares et al., 2004). The main aim was to answer two main questions; (i) What are the patterns of small mammal phylogenetic and functional diversity and community structure in Mt. Kenya and Chuylu hill's elevational gradients? and (ii) What are the main environmental predictors of small mammal phylogenetic and functional diversity and community structure in Mt. Kenya and Chuylu Hill's elevational gradients? Because there are hardly studies of Afromontane small mammals based on phylogenetic and functional diversity metrics, we expected patterns to generally conform to species richness observations from tropical mountains (Cisneros et al., 2014; Dreiss et al., 2015), studies of large African mammals (Kamilar et al., 2015; Rowan et al., 2016), or rodents (García-Navas, 2019), mostly due to high correlational associations. We expected stronger environmental filtering at extreme lower and upper elevations and stronger competitive filtering at the intermediate elevations as a legacy of habitat productivity and water-energy availability influences in tropical high mountains (Hawkins et al., 2003; Cardillo, 2011; Fisher et al., 2011; Kamilar et al., 2015; Torres-Romero and Olalla-Tarraga, 2015). Thus, we hypothesized that these extreme lower and upper elevation assemblages would be comprised of less closely related taxa than expected by chance (phylogenetic and functional overdispersion) while the assemblages in the intermediate/mid-elevations would be comprised of more closely related taxa than expected by chance (clustering) (Webb et al., 2002; Cavender-Bares et al., 2009; Gerhold et al., 2015). To test for the strongest abiotic drivers of diversity patterns, we tested various hypotheses implicated in the literature (Hawkins et al., 2003; McCain, 2005; McCain and Grytnes, 2010). Specifically, we explored (i) the water-energy availability hypothesis (Rosenzweig, 1992; Rahbek, 1995) to test whether the phylogenetic and functional diversity increases with increasing water availability and ambient energy, (ii) the primary productivity hypothesis (Rosenzweig, 1992; McCain et al., 2018) to test whether the phylogenetic and functional diversity increases with increasing habitat productivity, and (iii) the topographic heterogeneity hypothesis (Rosenzweig, 1995) to test whether the phylogenetic and functional diversity is positively correlated with topographic heterogeneity.

MATERIALS AND METHODS

Study Area and Sampling Design

The study was conducted in Mt. Kenya and the Chyulu Hills mountain range, hereon just "Chyulu." Mt. Kenya is an extinct stratovolcano that was formed between 3.1 and 2.6 million years ago (mya) and is Africa's second highest peak (5,199 m) next to Mt. Kilimanjaro (The World Heritage Committee of UNESCO, 2016). The south-eastern slope (Chogoria) is the windward side, which receives about 2,500 mm of average annual rainfall while the north-western slope (Sirimon) is the leeward side, which is comparatively drier than Chogoria, receiving about 800 mm average annual rainfall (Bennun and Njoroge, 1999). In Chogoria, mixed Afromontane rain forest bordering farmlands at the base is succeeded by Afromontane rainforest, bamboo forest, undifferentiated forest, and Montane Ericaceous vegetation toward the peak. The Sirimon slope supports two main vegetation types—Afromontane undifferentiated forests from the base to around mid-elevations and Montane Ericaceous vegetation from around the mid-elevations toward the peak (van Breugel et al., 2015; Musila et al., 2019a). Although Mt. Kenya is a global biodiversity hotspot (BirdLife International, 2012), the phylogenetic and functional diversity of small mammals in Mt. Kenya remains lacking. The Chyulu is a volcanic mountain range formed between 1.4 mya and the Holocene from a quaternary chain of volcanoes (Novak et al., 1997). It ranges in elevation from 1,000 m upland plains to 2,175 m at the peak. The region surrounding Chyulu is classified as arid and semiarid and receives about 400–500 mm of average annual rainfall. The peak elevations—around 2,000 to 1,800 m—is covered in "cloud" evergreen montane forest patches within grassland-shrub mosaics. The 1,000–1,200-m elevations are dominated by the Somalia-Masai Acacia-Commiphora bushlands and thickets while the 1,400–1,600 elevations are dominated by dry combretum wooded grasslands. Chyulu lacks any dedicated formal accounting of its small mammal biodiversity and the ecological patterns and processes. Both Mt. Kenya and Chyulu are global key biodiversity areas and important bird areas in Kenya (Bennun and Njoroge, 1999; World Database of Key Biodiversity Areas[1]). However, a few studies have focused on their mammalian community ecology, less so, on the small mammals. While the two highlands are in contrasting geographical localities (highland vs. lowland) and differ in peak elevations and aspect (steep vs. gentle), they are habitats to forest-dwelling small mammals with comparable ecologies and evolutionary baselines. The highest elevations of the Chyulu Hills serve as the northern frontier to the forest-dependent *Praomys delectorum* range while most of the Mt. Kenya small mammal fauna are forest specialists with ranges not extending to the lower less humid lowlands. Therefore, they comprise a good context to study elevational breakpoints in the diversity distributions and the contribution of abiotic conditions to such patterns (Graham et al., 2014).

We conducted a fieldwork from September 6, 2015 to October 13, 2015 in Mt. Kenya and September 8–22, 2016 in the Chyulu Hills. The sampling techniques targeted rodents and shrews based on the traps (Snap, Sherman, Pitfall buckets) and baits (raw peanut and oat flakes) used and transect and trapline setting. In Mt. Kenya, sampling was conducted in the windward side—Chogoria—where 43 traplines in nine transects were laid from ≈1,700–1,900 m (KC1800) to ≈3,300–3,500 m (KC3400), and the leeward side—Sirimon—where 45 traplines in 9 transects were laid from ≈2,300–2,500 m (KS2400) to ≈3,900–4,200 m (KS4000) (**Figure 1**). In the Chyulu Hills, sampling spanned a single slope from 1,000 to 2,000 m, with 18 traplines in 6 transects laid between ≈900–1,100 (CH1000) and ≈1,900–2,100 m (CH2000) (**Figure 1**). All transects were laid at ≈200-m intervals (**Figure 1**), and up to six traplines were surveyed at every transect. A trapline consisted of 20–30 trap stations set 10 m apart; each station included a Sherman trap, Snap trap, and Pitfall trap set 1–2 m apart. Traps were set around mid-mornings, checked in the mornings, and left at the same trapline for two nights before moving on to new transects. About 60–140 (average 110) trap nights were accumulated at every trapline in Chyulu, 50–66 (average 62) trap nights in Chogoria, and 22–74 (average 58) trap nights in Sirimon. A total of 2,578 specimens [Mt. Kenya: 1901 (Sirimon: 1008, Chogoria: 893), Chyulu: 677] after a total of 17,579 trap nights were sampled. We accumulated 4,859 trap nights in Chyulu at a 13.9% trap success and 12,720

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[1] http://www.keybiodiversityareas.org/kba-data

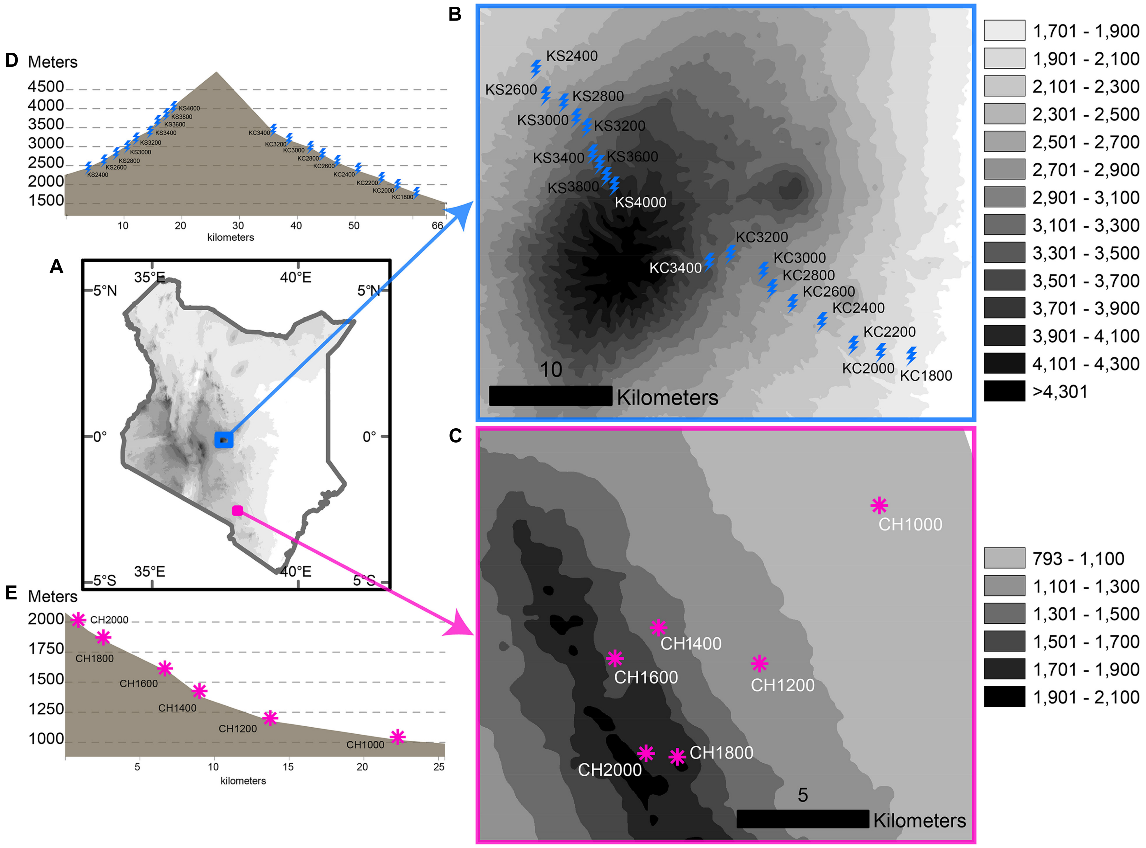

FIGURE 1 | Study area and field sampling design of this study. **(A)** Map of Kenya showing the location of Mount Kenya and Chyulu; **(B,C)** Sampled elevational transects in Mt. Kenya and Chyulu, respectively. **(D,E)** Vertical elevational profile vs. distance along the Mt. Kenya and Chyulu transects, respectively.

trap nights in Mt. Kenya at a 14.9% trap success [Chogoria 6,480 trap nights (13.8% trap success) and Sirimon 6,240 trap nights (16.2% trap success)].

The live-trapped animals were euthanized using chloroform after which the measurements of five external morphological traits (body mass in grams, head-body length, hind foot length, tail length, and ear length in centimeters) were recorded and the muscle and/or liver tissue extracted for later DNA analyses from all samples. Voucher specimens (skins and skulls) were also prepared for select specimens. The tissue samples and voucher specimens are deposited at the National Museums of Kenya, Nairobi, Kenya and Kunming Institute of Zoology, Kunming, China. All animal handling procedures adhered to the wildlife research laws of Kenya and the guidelines for the use of wild mammal species in research and education (Sikes and The Animal Care Use Committee of the American Society of Mammalogists, 2016). The research permit application was reviewed and approved by the Research and Ethics Committee of the Kenya Wildlife Service (permit number: KWS/BRM/5001).

Community Phylogenetic and Functional Characterization

For the input phylogenetic tree to estimate phylogenetic diversity indices, we used new sequences from this study to reconstruct a time-calibrated tree of all the unique gene pools (adopted as species units). For this, we extracted genomic DNA and sequenced the mitochondrial *Cytochrome b* gene from 1,142 samples. Recent evidence suggests *Cytochrome b* accurately reconstructs mammalian species' ordinal, familial, and generic phylogenetic associations that match currently recognized relationships from molecular and morphological data (Tobe et al., 2010). First, total DNA was extracted from the muscle and/or liver tissue preserved at −80°C in 99.6% ethanol, using the sodium dodecyl sulfate method (Sambrook et al., 1989). A 1,140 base-pair region of the *Cytochrome b* gene was successfully amplified using polymerase chain reaction (PCR) with the primers L14724 (5′–GGACTTATGACATGAAAAATCATCGTTG–3′) and H14139 (5′–GATTCCCCATTTCTGGTTTACAAGAC–3′) (Kocher et al., 1989). The PCR conditions included denaturation at 95.0°C for 30 s, annealing at 59.0°C for 30 s, and extension at 72.0°C for 60 s for 40 cycles. The amplified products were sequenced in forward and reverse directions using the same primers, and the resulting chromatograms *de novo* assembled in Geneious Prime® 2021.1.1[2] and aligned in Aliview based on the MUSCLE algorithm (Edgar, 2004; Larsson, 2014). The

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[2] https://www.geneious.com/

alignment was checked for internal stop codons and frameshifts in MEGA X (Kumar et al., 2018). From the 1,142 sequences, we identified 614 haplotypes using DnaSP V6.12.03 (Rozas et al., 2017). The haplotypes were retained as a new input alignment for reconstructing a preliminary phylogeny for species delimitation purposes (the haplotype data file was generated with sites with gaps or missing nucleotides and invariable sites included to retain the full 1,140 base-pair length). We also downloaded *Cytochrome b* sequences of the Numbat (*Myrmecobius fasciatus*), Brown-eared woolly opossum (*Caluromys lanatus*), and Water opossum (*Chironectes minimus*) from GenBank for use as an outgroup. For robustness and to check for topological inconsistencies of different phylogenetic methods, the preliminary phylogenetic tree was reconstructed using maximum likelihood in raxmlGUI 2.0 with 1,000 bootstrap replicates for branch support estimations (Stamatakis, 2014; Edler et al., 2021) and Bayesian inference in BEAST2 based on the uncorrelated lognormal clock and Yule speciation model with two MCMC runs each constituting 100 million MCMC chains sampled every 10 k runs and sampling convergence assessed in Tracer (Drummond and Rambaut, 2007; Bouckaert et al., 2019). In both analyses, we used the GTR + I + G4 nucleotide substitution model (identified as the best-fitting in ModelTest-NG v0.1.7; Darriba et al., 2019). We used the resulting trees and corresponding input alignments for species delimitation using tree- and distance-based methods capable of identifying common species units without prior sample assignment to taxonomic units. For the tree-based methods, we used the branch-cutting method (Ondřej, 2018), the multi-rate Poisson Tree Processes (mPTP) algorithm method (Kapli et al., 2017), and the single threshold general mixed Yule coalescent (GMYC) model (Fujisawa and Barraclough, 2013) while the distance-based method was implemented using assemble species by automatic partitioning (ASAP); (Puillandre et al., 2021).

The four delimitation methods differed in the number of OTUs estimated; ASAP yielded 49 OTUs, BCUT 49 OTUs, mPTP 58 OTUs, and GMYC 63 OTUs. The delimited OTUs were resolved into 63 clades, to capture all possible unique gene pools (populations or species); these were adopted as resembling currently recognized or potentially undescribed species pools (**Supplementary Material 1**). The clades' nucleotide sequences were submitted to GenBank, accession numbers OL408896–OL408958. To assign species names to the clades, we cross-referenced our initial taxonomic assignments in the field, morphological characterizations, and BLAST search matches with the latest mammal checklists—Musser and Carleton (2005), Dieterlen (2013), Monadjem et al. (2015), Denys et al. (2017), and Musila et al. (2019b). Sequences corresponding to the 63 clades were used to reconstruct the final time-calibrated tree as an input for estimating phylogenetic diversity dimensions. Divergences were estimated based on the most recent common ancestor (MRCA) calibrations. Date estimates were extracted from Kumar et al. (2017) and the cited references. Consequently, we defined four calibration points: (i) the MRCA of subfamily Deomyinae and Gerbillinae (for clades under *Acomys, Lophuromys,* and *Gerbilliscus* genera) at 24.9 (interval: 17.0—32.8) mya, (ii) the MRCA of clades under the Muridae, Nesomyidae, and Gliridae family at 71 (interval: 66–75) mya, (iii) the MRCA of the Soricidae family clades (*Crocidura, Sylvisorex,* and *Surdisorex* genera) at 34 (23–45) mya, and (iv) the MRCA of the outgroup taxa (*M. fasciatus, C. lanatus*, and *C. minimus*)—Infraclass Metatheria—at 82 (interval: 74–89) mya. The tree was reconstructed in BEAST2 using lognormal priors, uncorrelated lognormal clock, and Yule speciation model, two 100 million generations-long MCMC runs sampled every 10,000 runs. The sampling adequacies were assessed in Tracer, the trees and log files combined in LogCombiner after discarding 10% as burn-in, and the combined trees summarized with the maximum clade credibility method in TreeAnnotator (Drummond and Rambaut, 2007). The phylogenetic trees may be seen in **Supplementary Material 2**.

The input data set for estimating functional diversity indices was compiled from several traits representing species morphological characterizations, diet, and activity patterns recorded in the field and from the published data sets. The morphology traits were taken from the measurements recorded in the field—adult body mass, length of head + body, tail, hindfoot, and ear (**Supplementary Material 1**). The measurements were represented in the final trait data set by the first two axes, which together explained 95.6% variance (PC1: 83.3%, PC2: 12.3%) following the transformation using principal component analysis (PCA). We obtained the data on diet and activity patterns from EltonTraits (Wilman et al., 2014), PHYLACINE (Faurby et al., 2018), and MammalDIET (Gainsbury et al., 2018). The resulting diet traits included the proportions composed of plants, fruits, nectar, seeds, invertebrates, mammals and birds, fish, reptiles and amphibians, and scavenging (**Supplementary Material 1**). Because the scaling of species traits varied between the different data sets, we used the first two axes of PCAs as final diet variables (**Supplementary Material 1**). We also used the activity traits from Wilman et al. (2014) represented by binary scores of three levels (nocturnal, diurnal, or crepuscular). Overall, the morphological, diet, and activity traits relate to species' strategies of resource acquisition and usage underlying their life history and ecological strategies inherent in how biodiversity forms and is maintained in an area (Jones et al., 2009; Bohm et al., 2013). Functional diversity indices were estimated using the species-by-traits matrix or a functional trait dendrogram obtained by converting the matrix into a dendrogram of species relationships (Petchey and Gaston, 2002). The representation of species functional relationships using a dendrogram is easily applicable to and comparable with other biodiversity metrics using common frameworks, besides having a well-established historical background (Jarzyna and Jetz, 2016).

Compilation of Potential Abiotic Predictors

The environmental predictor data were extracted from publicly available repositories or following data requests from authors. To test the water-energy availability hypothesis, we used annual mean precipitation (PAM), annual precipitation seasonality (PAS), annual mean temperature (TAM), annual temperature seasonality (TAS), and annual actual evapotranspiration (AET).

We obtained TAM and TAS data for the near current climate data from the bioclimatic variables database (Fick and Hijmans, 2017), downloaded from https://www.worldclim.org/data/index.html. The AET data were extracted from Running et al. (2019); to control for anomalies associated with uneven monthly and yearly trends, we extracted 11 yearly AET products spanning 2009–2019 and averaged them into a single layer using the Raster Calculator tool in ArcMap. The AET was downloaded using the Application for Extracting and Exploring Analysis Ready Samples (AρρEEARS) (AppEEARS Team, 2020). To test the primary productivity hypothesis, we used normalized difference vegetation index (NDVI) and vegetation characterization (VEG). The NDVI data were extracted as the spectral index of vegetation reflectance in the near-infrared and red electromagnetic spectrum portions from Didan (2015), downloaded using AρρEEARS. For vegetation characterization data (VEG), we consolidated field-observed vegetation types [see Table 1 in Musila et al., 2019a and vegetation classification data from the *potential natural vegetation map of eastern Africa (extract for Kenya)* (van Breugel et al., 2015)].[3] The topographic heterogeneity hypothesis was explored using elevation (ELEV) and terrain ruggedness index (TRI). The ELEV data were obtained from the NASA Shuttle Radar Topography Mission 1 arc second data downloaded using AρρEEARS. Then, the TRI was derived from the ELEV layer using the Raster analysis "Terrain Ruggedness Index" function in QGIS (QGIS Development Team, 2021).

Estimation of Phylogenetic and Functional Indices

The increasing realization that species richness (SR) poorly surrogates functional and phylogenetic biodiversity dimensions, especially when life history and geographical specificities are factored, has led to increased adoption of phylogenetic and functional dimensions in the biodiversity field as a more holistic appraisal of conservation gaps and ecological restoration needs (Chao et al., 2014; Mazel et al., 2014; Tucker et al., 2017). We estimated six diversity indices representing the phylogenetic and functional sum of difference among observations—phylogenetic richness (PD) and functional richness (FD), average divergence among observations—phylogenetic mean nearest taxon distance (PD^{MNTD}) and functional mean nearest taxon distance (FD^{MNTD}), and variances of differences among observations (community structure)—phylogenetic nearest taxon index (PD^{NTI}) and functional nearest taxon index (FD^{NTI}). This approach ensured a more holistic characterization of the sampled sites. Because the PD and FD rely on species (taxonomic identities) distributions across functional and phylogenetic spaces, they are inherently correlated with species richness (Pavoine et al., 2013). On the other hand, the MNTD and NTI indices control for species richness non-independence by comparing observations to null model communities. Overall, these indices are broadly popular in the literature, which enhance the comparability of our results (Chao et al., 2014; Tucker et al., 2017).

For these implementations, the number of samples per clade (abundances) were first normalized using the Hellinger transformation (Legendre and Gallagher, 2001), which standardized the recorded clade numbers to sample totals by converting the absolute values to relative values. The transformation is suitable for species abundances as it gives low weights to variables with low counts and many zeros (Legendre and Gallagher, 2001). The three input data sets (site-by-clade community composition, phylogeny, and traits) and those of predictors were then sorted to match using the functions match.phylo.comm and match.phylo.data in R package *picante* (Kembel et al., 2010) and the *all.equal* function in the *base* package (R Core Team, 2021). The PD and FD were computed using the *ses.fd* function of *picante* with the phylogeny and functional dendrogram as the corresponding tree inputs. The PD^{MNTD} was estimated as the abundance-weighted estimates of evolutionary (phylogenetic) diversity using the *ses.mntd* function (Webb et al., 2002; Webb and Donoghue, 2005) in *picante*. Higher PD^{MNTD} values indicate that the sampled community is comprised of less related taxa. The FD^{MNTD} was estimated analogously as PD^{MNTD} after substituting the phylogenetic tree with the functional traits' dendrogram (Webb et al., 2002, 2008). The PD^{NTI} and FD^{NTI} represented the phylogenetic and functional community structure, respectively, with significantly positive values suggesting that communities are comprised of taxa that are more closely related than expected by chance while negative values suggest that communities are comprised of taxa that are less closely related than expected by chance. They were estimated from the standardized effect size of PD^{MNTD} and FD^{MNTD} by comparing with null-community expectations. The null communities were generated using 9,999 randomizations through 1,000 iterations based on the name-shuffling null model, which shuffles species names across the phylogenetic tree and the functional dendrogram/species-by-trait matrix. The resulting values were multiplied by -1 (negative 1) to translate them to PD^{NTI} and FD^{NTI} (Webb et al., 2008). The *number of taxa* that were also output from the above estimations was used to represent SR and explore correlations between the indices.

Multivariate Analysis

To align the predictors that were obtained as raster layers—PAM, PAS, TAM, TAS, AET, NDVI, ELEV, and TRI, we resampled (bilinear interpolation) them to a common 0.00277778 × 0.00277778-degree cell size using SDMtoolbox 2.0 in ArcMap (ESRI, 2011; Brown et al., 2017). We then extracted variables per sample using the Point sampling tool in QGIS and log-transformed the resulting values to normalize distributions (remove distribution skewness). To handle potential statistical and inferential issues associated with high multicollinearity (Graham, 2003), we retained single predictors from highly correlated sets [Pearson's r | > 0.7| (Dormann et al., 2013)]. We further used the variance inflation factor (VIF) analysis to handle overall predictors' non-independence, which might inflate standard errors when building regression models. For this, we used the generalized linear model (GLM), with the predictors as explanatory (independent) variables and each diversity index as response (dependent) variables. We used a VIF

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[3] https://vegetationmap4africa.org/

cut-off of three "3" (Zuur et al., 2010) at which predictors with VIFs greater than the cut-off were dropped, VIFs recalculated, and the procedure repeated until all predictors achieved the cut-off threshold. We used the *glmulti* R package (Calcagno, 2019) to find the best combination of predictors explaining diversity trends from all possible combinations of predictors, using main effects and the exhaustive screening option ranked by Akaike information criterion (AIC) scores. In several cases, there were more than one top best-fit models, which were not clearly distinguished from the top best model. Therefore, we averaged all models with changes in AIC scores (AIC^{Δ}) < 2 (Burnham and Anderson, 2004) using the R package *MuMIn* (Barton, 2019). The final global models were fitted with GLM and generalized additive model (GAM), which were preferred for providing data multidimensionality and non-linearity, as is characteristic of ecological relationships, with GAM concurrently used to automate the detection and estimation of non-linear effects (Hastie and Tibshirani, 1990; Guisan et al., 2002). The GLMs were fitted using the *stats* R base package (R Core Team, 2021) while GAMs were fitted using the *mgcv* R package with the thin plate regression splines (Wood, 2011). Because the responses (diversity indices) and explanatory variables (predictors) fitted normal distributions well, both models were fitted using the Gaussian probability distribution family and linear "identity" link. The proportions of diversity indices explained by the models and the relative importance of each predictor were estimated from the adjusted *r*-squared (r^2_{a}) and model coefficient. The bivariate correlations between predictors systematically differed between sites and slopes (**Supplementary Material 3**); therefore, analyses were implemented separately for the three elevational slopes (Chogoria, Sirimon, and Chyulu), and the contiguous Mt. Kenya data set (combining Chogoria and Sirimon). Consequently, we used 24 GLM and GAM models, each for the 6 diversity indices, with a similar number of models used to explore the individual predictive influence of elevation and vegetation on diversity variances.

RESULTS

Overview of Sampling and Diversity Distributions

The sample-size- (abundance-) based rarefaction and extrapolation curves of species sampling showed that transect sampling achieved asymptotes for all Hill diversity indices (**Supplementary Material 4**). The phylogenetic analyses recovered well-resolved associations at both deep and terminal divergences, with ordinal and familial relations matching currently recognized mammalian taxonomies (**Supplementary Material 2**). The samples were classified into 2 orders—Rodentia and Eulipotyphla, 4 families—Gliridae, Muridae, Nesomyidae, and Soricidae, 20 genera, and 63 clades (adopted as species units in the current study but not necessarily corresponding to currently recognized species) (**Supplementary Material 1**). Of the total 63 clades, 34 clades were represented in Mt. Kenya (31 clades in Chogoria and 23 clades in Sirimon) and 30 clades were represented in Chyulu.

On average, Chyulu was phylogenetically (PD) (333.3 ± 91.9 vs. 307.4 ± 78.3) and functionally (FD) (1.04 ± 0.28 vs. 1.01 ± 0.28) richer than Mt. Kenya. However, Mt. Kenya had a higher PD^{MNTD} (83.75 ± 23.54 vs. 61.8 ± 24.21) and FD^{MNTD} (0.255 ± 0.089 vs. 0.161 ± 0.075) compared to Chyulu. The PD^{NTI} and FD^{NTI} were both negative in Mt. Kenya (− 0.29 ± 0.94 and − 0.114 ± 0.0838, respectively) but positive in Chyulu (0.642 ± 1.102 and 0.75 ± 0.787, respectively) on average. Although fewer clades were represented in Sirimon, it had averagely higher PD and FD than Chogoria (317.71 ± 84.96 vs. 296.32 ± 69.75 and 1.063 ± 0.276 vs. 0.952 ± 0.285, respectively). Similarly, the PD^{MNTD} and FD^{MNTD} were also higher in Sirimon than in Chogoria (89.42 ± 20.52 vs. 77.67 ± 25.25 and 0.279 ± 0.082 vs. 0.231 ± 0.09, respectively). The PD^{NTI} and FD^{NTI} were both averagely negative in Sirimon (− 0.613 ± 0.857 and − 0.369 ± 0.723) but positive in Chogoria (0.057 ± 0.909 and 0.158 ± 0.875).

Associations between diversity indices were congruous between elevational gradients. The PD and FD increased with increasing SR while PD^{MNTD} and FD^{MNTD} decreased with increasing SR, all with statistically significant effects (**Supplementary Material 5**). On the other hand, PD^{NTI} and FD^{NTI} were not statistically significantly associated with SR, with PD^{NTI} increasing in Chyulu and Chogoria but decreasing in Sirimon as SR increased while FD^{NTI} decreased in Sirimon and Chogoria but increased in Chyulu as SR increased (**Supplementary Material 5**).

Phylogenetic and Functional Diversity Along Elevational Gradients

The predictive influence of elevation on diversity variances was observably not any better than that of the environmental variables retained in the best models or vegetation (**Table 1**). The strongest influence, based on the proportion of variance explained (r^2_{a}) was in Mt. Kenya (0.193) followed by Chogoria (0.095), Chyulu (0.0445), and the weakest in Sirimon (0.016). Between the indices, the strongest influence was on FD (r^2_{a} = 0.112) followed by PD^{MNTD}, PD, PD^{NTI}, FD^{MNTD}, and the weakest on FD^{NTI} (r^2_{a} = 0.066) (**Table 1**).

The richness indices (PD and FD) were positively associated with elevation in Mt. Kenya, where they portrayed a unimodal hump-shaped curve—mid-elevation peak—around 3300 m in the former (**Figure 2** and **Table 2**). Between the Mt. Kenya slopes, the PD and FD increased in Chogoria up to around 2,800 m and then declined, while in Sirimon both the indices non-significantly declined multimodally; with the two curves combining into the hump-shaped curve in Mt. Kenya (**Figure 2**). Both PD and FD unimodally increased with elevation in Chyulu, with no well-defined peaking around mid-elevations (**Figure 2** and **Table 2**).

The divergence indices (PD^{MNTD} and FD^{MNTD}) portrayed weaker elevational trends—near-horizontal response curves—compared to PD and FD in Mt. Kenya unlike in Chyulu (**Figure 2**). In Mt. Kenya, PD^{MNTD} and FD^{MNTD} increased with elevation, although non-significantly, indicating that phylogenetic and functional relationships between clades declined toward upper elevations overall. In contrast, different

TABLE 1 | Summary of best-fit model coefficients across diversity indices and sites.

		Model predictors							Elevation		Vegetation
Index	**Site/Slope**	**AET**	**NDVI**	**PAM**	**TAS**	**TRI**	**GLM R^2**	**GAM R^2**	**GLM R^2**	**GAM R^2**	**GLM R^2**
PD	Mt. Kenya		−151.28|| 29%	−295.77 *|| 71%			0.069	0.154	0.171	0.119	0.108
	Chyulu				1214.51|| 100%		0.178	0.127	0.045	0.09	0.117
	Chogoria		−350.93|| 33%		−127.89|| 34%	−142.22|| 33%	0.117	0.251	0.155	0.171	0.32
	Sirimon				93.09|| 10%	130.94|| 90%	0.019	0.185	0.003	0.283	0.002
FD	Mt. Kenya		−1.02|| 25%	−1.71 ***|| 70%		−0.14|| 5%	0.17	0.29	0.034	−0.027	0.021
	Chyulu				1.43|| 100%		0.027	−0.034	0.1	0.204	0.24
	Chogoria		−1.69|| 42%		−0.94|| 49%	−0.43|| 9%	0.207	0.379	0.3	0.328	0.459
	Sirimon			−0.8|| 38%		0.66|| 62%	0.041	0.257	0.015	0.337	0
PD^{MNTD}	Mt. Kenya			34.6|| 8%	51.21|| 33%	42.35 *|| 59%	0.093	0.255	0.314	0.637	0.387
	Chyulu	278.79 *|| 57%	264.59|| 37%		−85.39|| 6%		0.601	0.742	0.002	0.04	0.092
	Chogoria		282.27 *|| 55%		−73.23|| 7%	76.68 *|| 38%	0.183	0.596	0.055	0.076	0.205
	Sirimon			43.39|| 40%	62.19|| 29%	25.51|| 31%	0.054	−0.014	0.02	−0.002	0
FD^{MNTD}	Mt. Kenya				0.16|| 22%	0.18 *|| 78%	0.095	0.274	0.264	0.37	0.333
	Chyulu	0.94 *|| 83%			−0.54|| 17%		0.415	0.336	0.009	−0.002	0.069
	Chogoria		0.75|| 22%		−0.23|| 5%	0.33 **|| 73%	0.186	0.475	0.001	0.398	0.161
	Sirimon				0.22|| 100%		0.012	0.028	0.004	−0.019	0.032
PD^{NTI}	Mt. Kenya		1.99|| 37%		−1.91|| 20%	−1.39|| 44%	0.128	0.202	0.245	0.6	0.369
	Chyulu	−17.37 ***|| 100%					0.528	0.558	0.064	0.053	0.082
	Chogoria				0.96|| 48%	−0.54|| 52%	0.016	0.029	0.003	−0.022	0.057
	Sirimon			−1.19|| 20%	−3.11|| 18%	−2.68|| 62%	0.117	0.312	0.021	0.297	0.013
FD^{NTI}	Mt. Kenya			4.07 **|| 47%	−1.67|| 10%	−1.59 *|| 43%	0.166	0.218	0.13	0.27	0.23
	Chyulu	−9.41 *|| 100%					0.304	0.26	0.046	0.059	0.164
	Chogoria				1.5|| 35%	−1.18|| 65%	0.063	0.226	0.053	0.268	0.201
	Sirimon			2.62|| 34%	−3.16|| 39%	−1.67|| 27%	0.075	0.357	0.033	0.397	0.04

*The effect size of the generalized linear model (GLM) is shown on the left of the double vertical bars and the relative importance of each predictor is shown on the right. The singular influence of elevation and vegetation is shown for comparison. The effect sizes based on generalized additive models (GAM) is also shown wherever relevant. Mt. Kenya (n = 87); Chyulu (n = 19); Chogoria (n = 42); Sirimon (n = 45). Statistical significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.*

divergence trends were observed in Sirimon and Chogoria where both the indices decreased with elevation except for FD^{MNTD} in Sirimon. Both PD^{MNTD} and FD^{MNTD} declined statistically significantly with elevation in Chyulu (**Figure 2** and **Table 2**).

The community structure indices (PD^{NTI} and FD^{NTI}) also showed weak associations with elevation, with $p > 0.05$ except for PD^{NTI} in Mt. Kenya and Chyulu (**Figure 3A**). Both PD^{NTI} and FD^{NTI} declined with increasing elevation in Mt. Kenya, Chogoria, and Sirimon but increased in Chyulu and Sirimon (FD^{NTI} only) (**Figure 3A**). Thus, the tendency of assemblages to be phylogenetically and functionally overdispersed (less closely related than expected by chance) increased with elevation in Mt. Kenya while clustering (more closely related than expected by chance) increased with elevation in Chyulu (**Figure 3B**). Overall, assemblages were randomly phylogenetically and functionally structured (of 105 traplines, 98 had PD^{NTI} with $p > 0.05$ and 103 for FD^{NTI}).

Environmental Predictors of Diversity Variances

Only four predictors were retained across the best models; annual mean precipitation (PAM), temperature seasonality (TAS), annual actual evapotranspiration (AET), normalized difference vegetation index (NDVI), and terrain ruggedness index (TRI) (**Figure 4A** and **Table 1**). The proportions of diversity variances accounted for by the best models (r^2_a) differed between the sites and indices, averaging 0.161 (0.096 − 0.233) and the highest in Chyulu (0.342) followed by Chogoria (0.129), Mt. Kenya (0.12), and the least in Sirimon (0.053) (**Figure 4B** and **Table 1**). Observably, no single index was consistently similarly explained (**Figure 4A** and **Table 1**). Also, the GAMs were notably consistently better at explaining associations than GLMs (**Figure 4B** and **Table 1**).

The response curves of associations between diversity variances and the strongest predictors across the sites and indices are illustrated in **Supplementary Material 6**. In Mt. Kenya, annual mean precipitation (PAM) and terrain ruggedness index (TRI) were the most important predictors. The PAM explained the biggest relative proportion of the r^2_a in PD, FD, and FD^{NTI} while TRI accounted for the biggest proportion of the PD^{MNTD}, FD^{MNTD}, and PD^{NTI} variances (**Figure 4A** and **Table 1**). Notably, the most important predictors in Chogoria and Sirimon differed from those of Mt. Kenya, with TAS, NDVI, and TRI as the strongest predictors (**Table 1**). In Chogoria, the lowest r^2_a, which was in PD^{NTI} ($r^2_a = 0.016$), was explained mostly by TRI, followed by FD^{NTI} ($r^2_a = 0.063$) which was also explained mostly

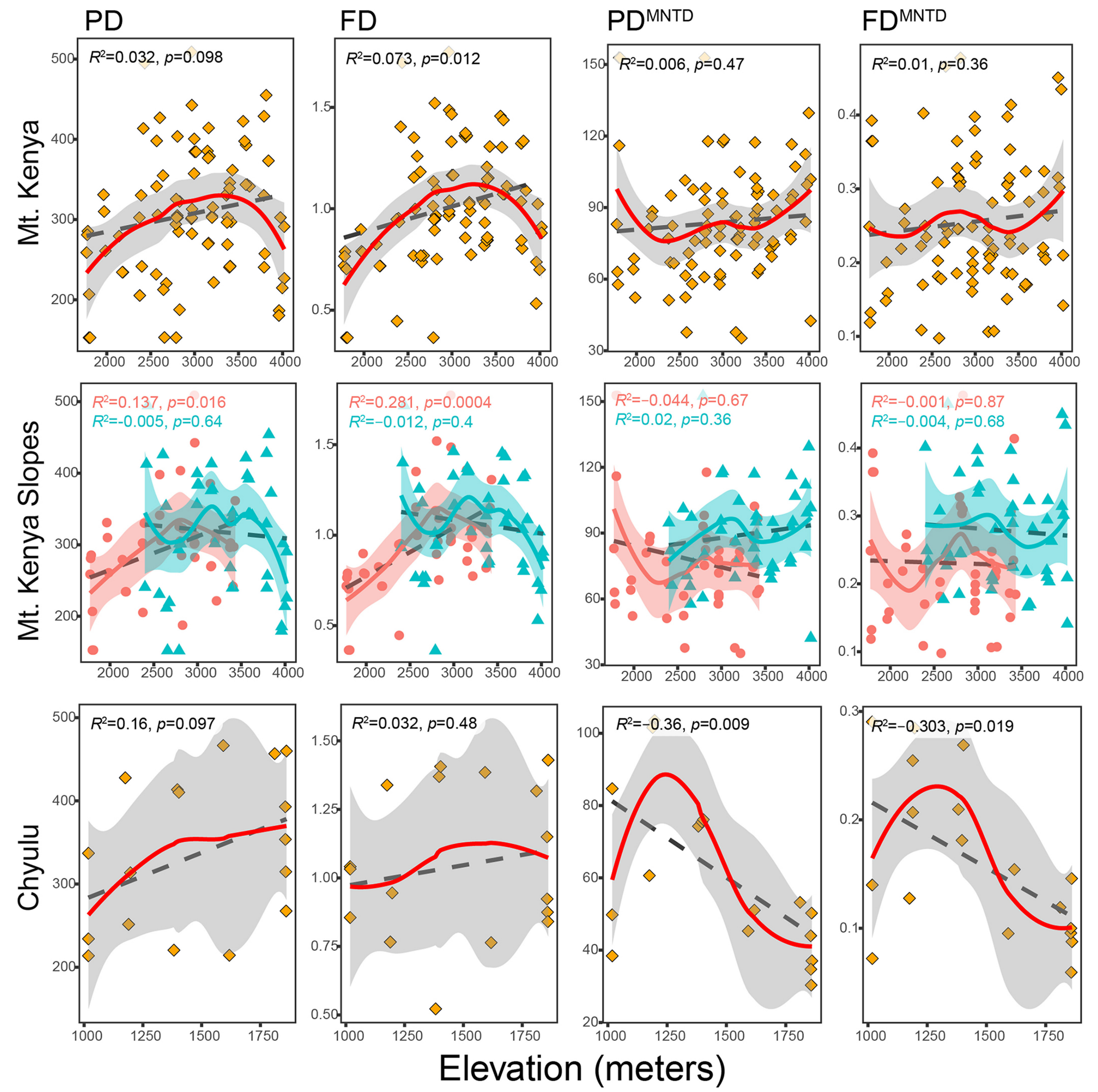

FIGURE 2 | The distribution of phylogenetic and functional diversity and divergence indices (*y*-axes) along elevational gradients (*x*-axes) between the sites and indices. Points represent traplines and lines represent regression lines—local polynomial (solid colored lines) and generalized linear model (GLM; black dashed lines). In the Mt. Kenya plots, light red-colored dots represent Chogoria traplines while the dark shade of cyan triangular points represents Sirimon traplines.

by TRI, PD (r^2_a = 0.117) by annual temperature seasonality (TAS), PD^{MNTD} (r^2_a = 0.183) by the normalized difference vegetation index (NDVI), FD^{MNTD} (r^2_a = 0.186) by TRI, and the highest in FD (r^2_a = 0.207) which was mostly correlated with TAS. On the other hand, the explained diversity variances in Sirimon ranged between 0.012 and 0.117 and were mostly correlated with PAM, TAS, and TRI. The PAM was the strongest predictor of the 0.054 r^2_a of PD^{MNTD} with TAS explaining most of the 0.012 r^2_a of FD^{MNTD} and 0.075 r^2_a of FD^{NTI} and TRI explaining most of the 0.019 r^2_a of PD, 0.041 r^2_a of FD, and 0.117 r^2_a of PD^{NTI} (**Figure 4B** and **Table 1**). In Chyulu, AET and TAS were the strongest predictors of the 0.027–0.601 r^2_a across the indices. The AET explained most of the 0.601 r^2_a of PD^{MNTD}, 0.415 r^2_a of FD^{MNTD}, 0.528 r^2_a of PD^{NTI}, and 0.26 r^2_a of FD^{NTI} while TAS accounted for most of the 0.178 r^2_a of PD and 0.027 r^2_a of FD (**Figure 4B** and **Table 1**).

Despite the relatively well-variegated vegetation distribution along elevational gradients (**Supplementary Material 7**), its

TABLE 2 | Summary statistics of diversity indices between the transects.

Site	Transect	SR	PD	FD	PD$^{\text{MNTD}}$	FD$^{\text{MNTD}}$	PD$^{\text{NTI}}$	FD$^{\text{NTI}}$	N
Chyulu	CH1000	6.3 ± 1.5	261.60 ± 66.09	0.976 ± 0.105	57.62 ± 24.12	0.167 ± 0.112	0.968 ± 1.046	0.822 ± 1.056	18
	CH1200	5.5 ± 2.4	311.09 ± 83.07	0.954 ± 0.270	91.97 ± 20.91	0.218 ± 0.068	−0.537 ± 0.554	0.401 ± 0.511	
	CH1400	7.0 ± 2.6	348.06 ± 110.48	1.100 ± 0.500	75.29 ± 0.94	0.220 ± 0.045	−0.306 ± 0.759	−0.139 ± 0.956	
	CH1600	8.5 ± 5.0	340.44 ± 178.28	1.075 ± 0.439	48.17 ± 4.08	0.125 ± 0.042	1.241 ± 0.299	1.151 ± 0.080	
	CH1800	11.3 ± 1.5	436.47 ± 37.79	1.299 ± 0.141	44.74 ± 8.11	0.101 ± 0.016	1.143 ± 0.577	1.101 ± 0.288	
	CH2000	8.7 ± 2.5	311.99 ± 43.12	0.880 ± 0.042	38.42 ± 10.47	0.102 ± 0.043	1.936 ± 0.543	1.414 ± 0.565	
	F|| *p*	2.171|| 0.126	1.339|| 0.313	0.818|| 0.560	6.012|| 0.005	2.335|| 0.106	6.450|| 0.004	2.076|| 0.139	
Chogoria	KC1800	4.0 ± 1.9	222.91 ± 61.12	0.618 ± 0.198	104.20 ± 42.79	0.270 ± 0.123	−0.193 ± 0.796	0.433 ± 0.688	42
	KC2000	6.7 ± 0.6	300.61 ± 35.94	0.825 ± 0.064	61.63 ± 8.43	0.169 ± 0.028	0.559 ± 0.595	0.639 ± 0.411	
	KC2200	4.3 ± 0.6	249.31 ± 25.99	0.754 ± 0.062	85.25 ± 3.82	0.248 ± 0.027	−0.015 ± 0.057	0.290 ± 0.132	
	KC2400	5.0 ± 1.0	279.27 ± 65.55	0.782 ± 0.291	65.08 ± 13.05	0.167 ± 0.057	0.690 ± 0.753	0.905 ± 0.647	
	KC2600	7.7 ± 1.2	348.34 ± 46.58	1.168 ± 0.177	50.50 ± 11.99	0.161 ± 0.056	1.123 ± 0.721	0.555 ± 0.823	
	KC2800	5.5 ± 1.6	304.87 ± 70.19	1.060 ± 0.264	92.34 ± 14.78	0.341 ± 0.067	−0.663 ± 0.308	−0.987 ± 0.469	
	KC3000	8.0 ± 2.1	358.36 ± 93.82	1.203 ± 0.343	68.79 ± 10.00	0.197 ± 0.031	−0.145 ± 0.921	−0.059 ± 0.875	
	KC3200	6.0 ± 1.1	294.43 ± 55.33	0.991 ± 0.203	65.93 ± 23.06	0.187 ± 0.063	0.435 ± 1.419	0.543 ± 0.873	
	KC3400	5.7 ± 1.2	304.92 ± 39.87	1.030 ± 0.185	81.20 ± 16.72	0.246 ± 0.099	−0.212 ± 0.727	−0.016 ± 1.018	
	F|| *p*	3.982|| 0.002	2.331|| 0.042	3.652|| 0.004	2.781|| 0.018	3.130|| 0.010	1.828|| 0.107	2.750|| 0.019	
Sirimon	KS2400	7.7 ± 3.5	383.44 ± 129.46	1.353 ± 0.395	81.32 ± 14.24	0.296 ± 0.093	−0.683 ± 0.351	−1.160 ± 0.407	45
	KS2600	5.0 ± 2.0	266.55 ± 102.62	0.914 ± 0.239	78.32 ± 17.49	0.278 ± 0.101	0.203 ± 0.682	−0.055 ± 0.571	
	KS2800	4.5 ± 1.7	262.92 ± 73.55	0.819 ± 0.304	93.41 ± 40.58	0.260 ± 0.075	−0.068 ± 0.671	0.255 ± 0.194	
	KS3000	6.3 ± 0.5	381.69 ± 17.95	1.282 ± 0.145	105.85 ± 14.06	0.364 ± 0.030	−1.806 ± 0.690	−1.643 ± 0.442	
	KS3200	7.5 ± 1.7	368.08 ± 46.95	1.278 ± 0.170	84.46 ± 15.20	0.223 ± 0.040	−0.902 ± 0.622	−0.204 ± 0.215	
	KS3400	5.2 ± 1.3	294.73 ± 49.89	1.017 ± 0.157	93.59 ± 13.86	0.315 ± 0.058	−0.689 ± 0.536	−0.634 ± 0.453	
	KS3600	7.3 ± 1.0	373.57 ± 35.20	1.242 ± 0.181	79.37 ± 10.21	0.222 ± 0.067	−0.617 ± 0.505	−0.188 ± 0.870	
	KS3800	6.0 ± 1.8	347.48 ± 87.95	1.072 ± 0.226	94.61 ± 15.57	0.253 ± 0.049	−1.054 ± 0.783	−0.164 ± 0.199	
	KS4000	4.2 ± 1.3	233.66 ± 52.01	0.798 ± 0.175	94.99 ± 29.82	0.309 ± 0.121	−0.251 ± 1.168	−0.080 ± 0.798	
	F|| *p*	2.907|| 0.013	3.405|| 0.005	4.257|| 0.001	0.952|| 0.488	1.657|| 0.143	3.113|| 0.009	4.910|| 0.000	
	Total *F*|| *p*	3.616|| 0.000	2.464|| 0.002	3|| 0.000	3.242|| 0.000	3.522|| 0.000	4.488|| 0.000	4.626|| 0.000	

Corresponding statistical tests for the comparisons are provided by the site.
SR = species richness, PD = phylogenetic richness, FD = functional richness, PD^{MNTD} = phylogenetic mean nearest taxon distance, FD^{MNTD} = functional mean nearest taxon distance, PD^{NTI} = phylogenetic nearest taxon index, FD^{NTI} = functional nearest taxon index; F = F statistics; p = p-value; N = number of traplines.

singular influence on diversity variances remained low (**Table 1**). The r^2_a of vegetation ranged between 0 and 0.459 across the sites and indices (**Table 1**). Across the indices, the highest r^2_a was in Mt. Kenya (0.241), followed by Chogoria (0.234), Chyulu (0.127), and the least in Sirimon (0.014). The influence of vegetation among the indices did not greatly differ, with the r^2_a range of 0.13–0.18; being the strongest for FD and the weakest for PD$^{\text{NTI}}$ (**Table 1**).

DISCUSSION

The Afrotropical realm holds the highest mammal diversities globally, which are also most at the risk of extinction (Ceballos and Ehrlich, 2006; Jenkins et al., 2013; Raven et al., 2020). However, there are very few studies on local-scale ecosystem species mechanisms, such as elevational patterns in ecologies and evolutionary histories, with hardly any studies based on phylogenetic and functional dimensions. Here, we sought to answer the two main questions inherent in understanding mammal-environment feedbacks—what are the patterns of small mammal phylogenetic and functional diversity and community structure along elevational gradients and what are the main environmental predictors of small mammal phylogenetic and functional diversity and community structure along elevational gradients? Based on a comprehensive elevational sampling of Mt. Kenya and the Chuylu Hills mountain range, our analyses offer a first-of-its-kind insight into how the phylogenetic and functional diversity of small mammals vary along tropical mountain elevational gradients and how patterns are associated with environmental predictors.

Our first aim was to untangle the phylogenetic and functional diversity and community structure trends along the elevational gradients of Mt. Kenya and the Chyulu Hills and draw comparisons with species richness (SR), which has dominated the literature on diversity-elevation associations. We observed a hump-shaped PD and FD association as elevation increased in Mt. Kenya (combining the Chogoria and Sirimon slopes),

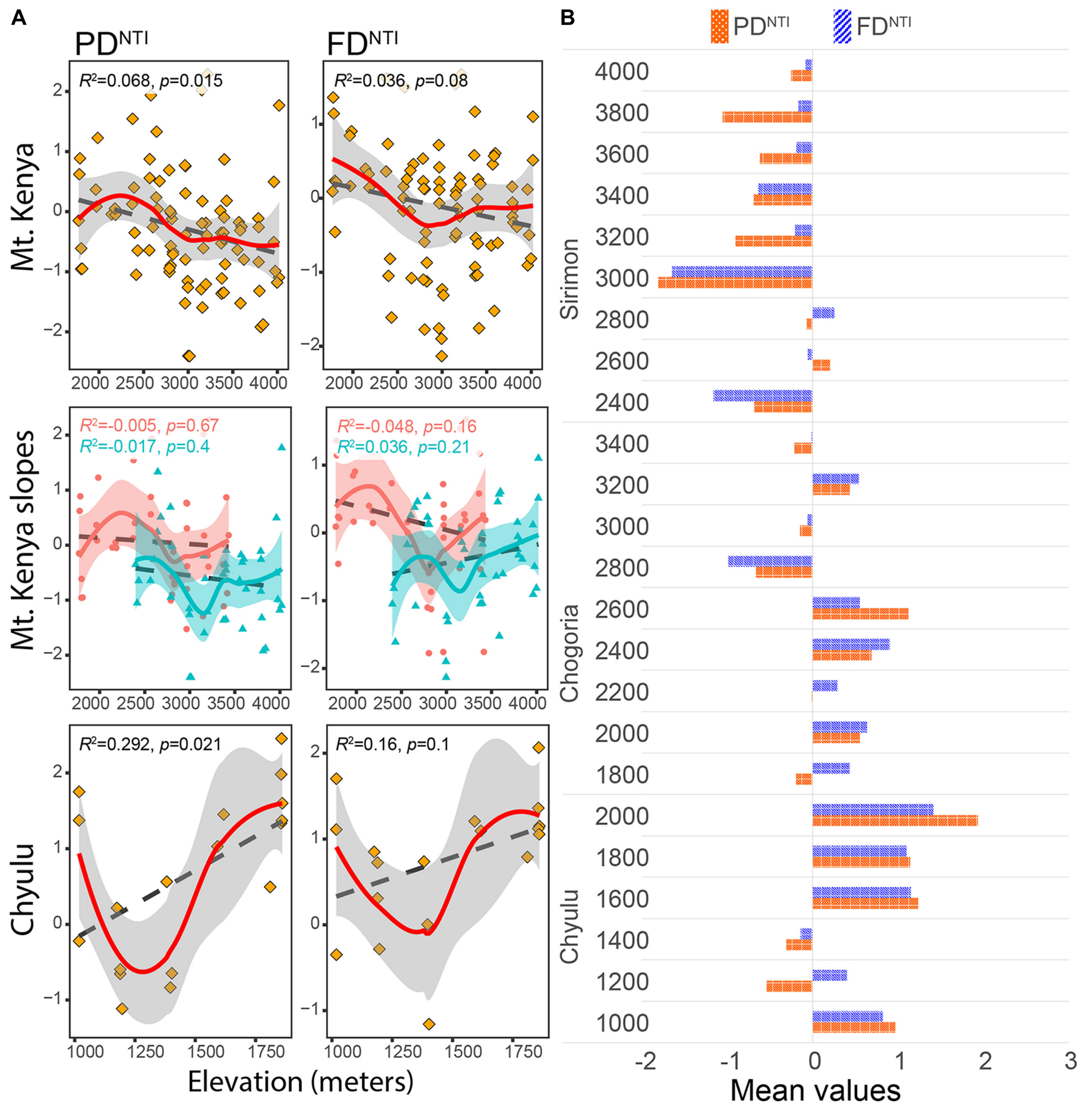

FIGURE 3 | The distribution of phylogenetic and functional community structure indices along elevational gradients in Mt. Kenya and the Chyulu Hills. **(A)** The association of community structure (*y*-axes) with elevation (*x*-axes) between elevational gradients. **(B)** The distribution of mean structure indices between the transects. Points represent traplines and lines represent the local polynomial (colored continuous lines) and GLM (black dashed line) regression lines. In the Mt. Kenya plots, light red-colored dots represent Chogoria traplines while the dark shade of cyan triangular points represents Sirimon traplines.

with Chogoria portraying a nearly similar curve. On the other hand, in Sirimon, the PD and FD were multimodally associated with elevation with no definite peak(s) while in Chyulu, the PD and FD unimodally increased with elevation. While the Mt. Kenya and Chogoria trends conform to a typical SR distribution in tropical mountains (McCain and Grytnes, 2010), the differing trends observed in Sirimon and Chyulu trends have also been recorded. In an affiliated Mt. Kenya study, Musila et al. (2019a) observed the highest number of small mammals' species around mid-high elevations in Chogoria but little elevational variation in Sirimon. Due to a high correlation of SR with PD and FD (**Supplementary Material 5**), the hump-shaped PD and FD association with elevation can be considered to be well supported by previous studies of mammalian assembling in tropical mountains (Rahbek, 1995; McCain, 2005; Chen et al., 2017; Shuai et al., 2017). Notably, however, these results contrast the Dreiss et al. (2015) study of neotropical rodents, the PD and FD declined monotonically as elevation increased. Also, Dehling et al. (2014) observed functional richness and phylogenetic diversity decreased monotonically with increasing elevation in the Andes while Zhou et al. (2019) study of phylogenetic and functional diversity of the Mt. Kenya seed plants was also dominated by PD and FD declines as elevation increased. These contrasting observations highlight the potential impacts of context-specific differences (animal group, mountain locality, or elevational gradient), which are even evident in the slope-wise—Chogoria vs. Sirimon—abiotic characterization (**Figure 5**). Although mid-elevation peaking in the PD and FD

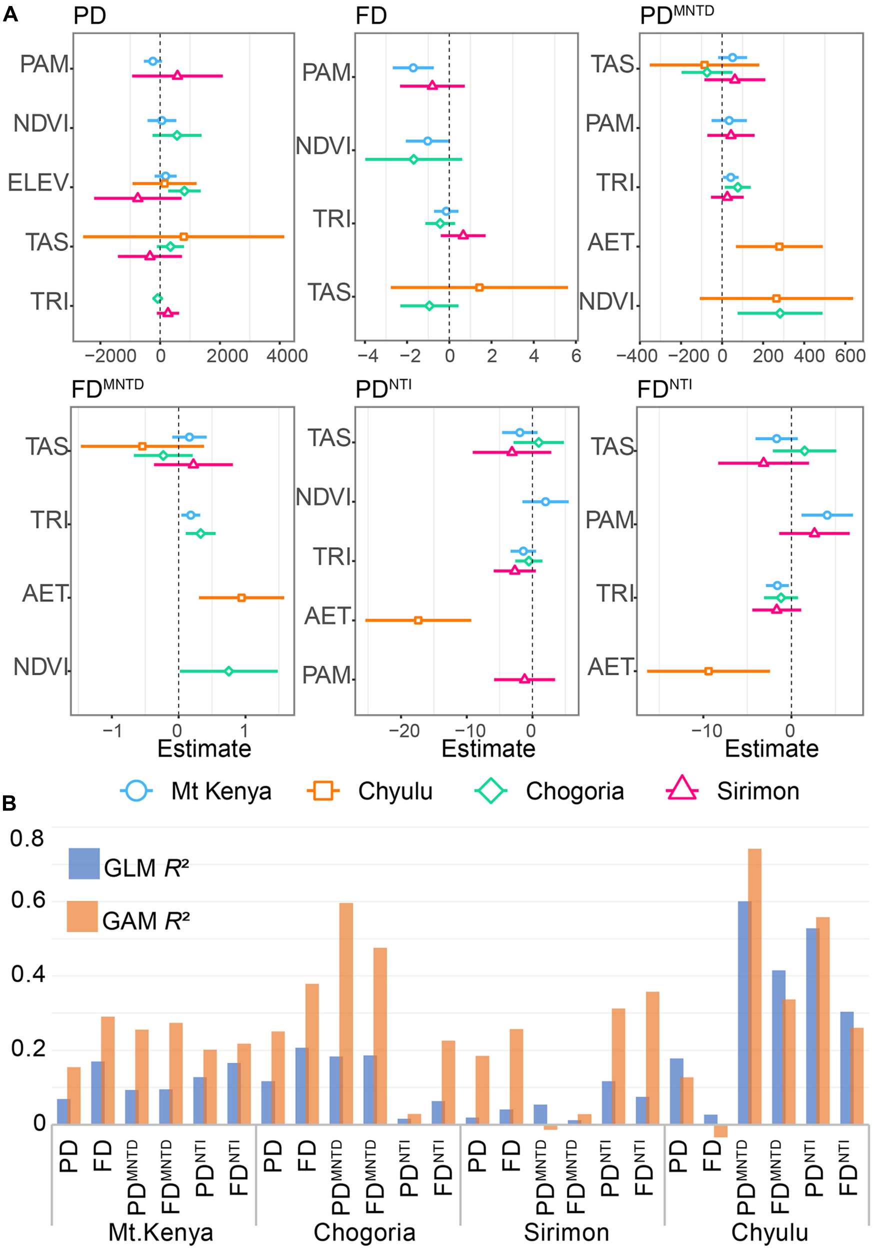

FIGURE 4 | The regression coefficients of association between diversity variances and environmental predictors. **(A)** Plots of regression coefficients summarizing the association between predictors and indices between the sites. **(B)** The proportion of diversity variances accounted for by the combination of predictors retained in the best-fit models.

(as a surrogate of SR) can dominate low-altitude mountains in vertebrate assemblages (Nogués-Bravo et al., 2008; Weier et al., 2016; Quintero and Jetz, 2018), the trends observed here in Chyulu imply that these low elevation highlands portray a broader array of diversity-elevation patterns. Also, the Chyulu Hills has much gentler slopes with broader and less definite base elevations and an overall harsher environment as most transects are characterized as arid and semiarid, which eliminates the formation of distinct mountain fauna for elevational trends. Studies generalizing elevation-diversity associations should also note environmental characterizations of the mountains for greater insights into local assembly dynamics in small mammal communities.

The increase of PD^{MNTD} and FD^{MNTD} with elevation in Mt. Kenya, Chogoria, and Sirimon relays a tendency of decreasing phylogenetic and functional affinity with increasing elevation. Such patterns are commonly attributed to the harsher habitats at higher elevations (such as night-freezing temperatures and resource-poor conditions in Mt. Kenya), which depress speciation and generational turnover for most small mammals,

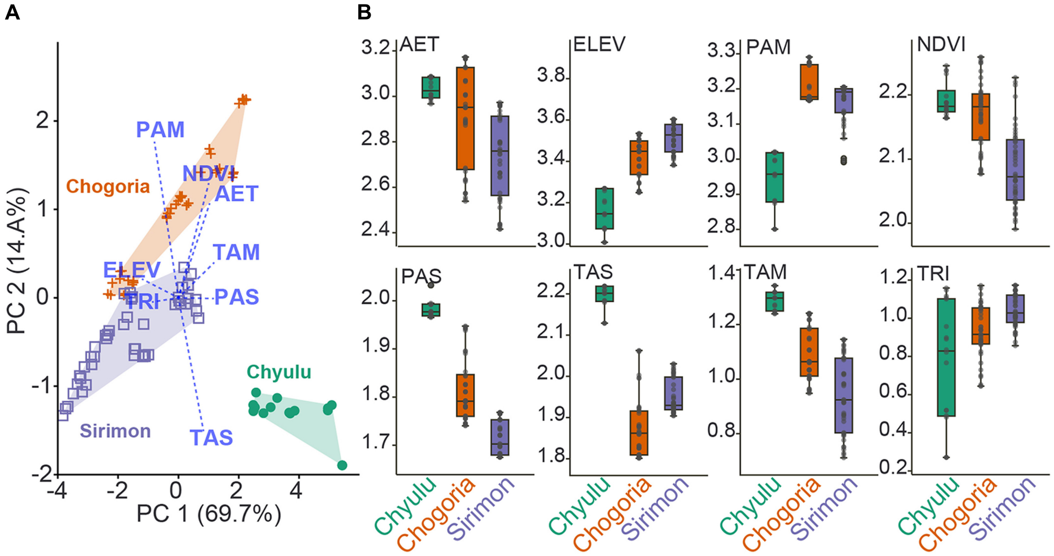

FIGURE 5 | The environmental characterization of Mt. Kenya and Chyulu based on the predictors used in the study. **(A)** Scatter plot of the first two principal component analysis (PCA) axes illustrating differences between elevational gradients. **(B)** Boxplots comparing individual predictor averages between the sites.

in the process, leading to the persistence of a few taxa uniquely adapted species (Hawkins et al., 2003; Graham et al., 2014). The clades sampled at the highest elevations in Mt. Kenya, besides the locally abundant ones such as clades in the genera *Lophuromys* and *Crocidura* (Musila et al., 2019a; Onditi et al., 2021), included *Dendromus, Otomys, Dasymys,* and *Rhabdomys* clades that were overall rarer at transects in lower elevations (**Supplementary Materials 1, 4**) and noted to have evolved to prefer the moist highland forests (Carleton and Musser, 2013; Denys et al., 2017). In Chyulu, such specialists included clades in the genera *Grammomys*, *Otomys*, *Praomys*, and *Zelotomys,* which were not recorded below the 1,800- and 2,000-m transects that had patches of Afromontane rainforests. These clades are only known from humid habitats, including highland forests, forest edges, and swamps (Monadjem et al., 2015; Denys et al., 2017; Musila et al., 2019b). The narrow transect ranges they were recorded in this study suggest they might be occurring in much narrower elevational ranges than is currently recorded, leading to pronounced influences on the phylogenetic and functional trends along elevational gradients. In Chyulu, PD^{MNTD} and FD^{MNTD} steeply declined as elevation increased, this can be attributed to higher elevational transects comprising more species that are closely related, i.e., forest-dependent clades cited above, compared to the lower elevational transects, which were dominated by a few clades that are common in more arid habitats—*Acomys* (not recorded above 1,600 m) and *Gerbilliscus* (not recorded above 1,600 m). This is also supported by a significant increase in PD^{NTI} with elevation, supporting that the likelihood of assemblages being clustered (comprised of the clades that are phylogenetically more related than expected by chance) increased with decreasing elevation. Such patterns typically manifest the influence of strong environmental filtering (Webb, 2000), which herein reflects the less productive habitats (Somalia-Masai Acacia-Commiphora bushlands and thickets and dry combretum wooded grasslands) that dominated the 1,200- and 1,000-m transects. The non-significant deviations of PD^{NTI} and FD^{NTI} from null models appear common when these indices are estimated from the standardized effect size of taxon distances across mammal, avian, herptile, and floral studies (Cisneros et al., 2014; Dehling et al., 2014; Dreiss et al., 2015; Meza-Joya and Torres, 2016; Zhou et al., 2019; Montaño-Centellas et al., 2020).

Our second main question pertained to the environmental predictors of small mammal phylogenetic and functional diversity and community structure along elevational gradients. Here, we tested three main hypotheses (water-energy availability, primary productivity, and topographic heterogeneity) implicated in the literature as strong mammal diversity drivers in tropical ecosystems (Hawkins et al., 2003; McCain, 2005; McCain and Grytnes, 2010). Considering the retention of AET, PAM, and TAS as the most important predictors of diversity variances across most of the models, our results notably strongly support the water-energy availability hypotheses (Rosenzweig, 1992; Rahbek, 1995) (**Figure 4** and **Table 1**). These predictors represent access to water resources, which are major limiting factors for mammals, especially in warmer tropical climates (Hawkins et al., 2003), and their importance as a key diversity and assembly predictor in Mt. Kenya conforms to these influences. Temperature and precipitation are also strong diversity drivers in other vertebrate and floral groups (Kessler and Kluge, 2008; Hanz et al., 2019; Ramirez-Bautista and Williams, 2019; Zhou et al., 2019; Rowan et al., 2020), African mammal communities (Kamilar et al., 2014, 2015), and plant–animal diversity and interactions in Mt. Kilimanjaro (Schellenberger Costa et al., 2017; Albrecht et al., 2018), as observed here for small mammals. Notably, AET and TAS were retained as strongly correlated with diversity variances mostly in Chyulu (**Table 1**), suggesting that their influence on small mammal assembling might be stronger in dominantly less moist habitats. The Chyulu Hills and Mt. Kenya are well differentiated based on the predictors, with Chogoria and Sirimon also observably differing for some predictors (**Figure 5** and **Supplementary Material 3**). The Mt. Kenya–Chyulu differences are supported by the previous studies that observed higher diversity variances explained by environmental variables in less productive/harsher ecosystems (Schluter and Pennell, 2017; Harvey et al., 2020), with harshness interpreted as higher TAS and PAS and lower PAM and AET. On the other hand, the Chogoria-Sirimon contrasts probably relate to aspect-wise variations in precipitation and temperature of tropical mountains, which has been observed to be strongly associated with small mammal diversity distributions in tropical mountains (McCain, 2007; Curran et al., 2012; Weier et al., 2016).

Although our results suggest that the habitat productivity hypothesis (Rosenzweig, 1992; McCain et al., 2018) might be a weaker predictor of PD and FD variances along elevational gradients in the tropics, the AET, PAM, and TAS control of primary habitat productivity, in terms of vegetation diversity and abundance (Hawkins et al., 2003), which has more direct influences on small mammal ecological strategies and the association may confound the predictive influence of NDVI and vegetation types on diversity variances. Notably, the retention of TRI as the strongest contributor to model-explained diversity variances in 9 of the 24 models supports the topographic heterogeneity hypothesis (Rosenzweig, 1995) as a strong regulator of how species assemble and interact based on their phylogenetic and functional characterizations in tropical mountains. Despite a few studies focusing on TRI's association with vertebrate elevational diversity trends on Afrotropical mountains, topographic heterogeneity at local habitat level scales is a significant regulator of primary habitat diversity and productivity (Stein and Kreft, 2015). This pertains to TRI and elevation being intrinsic regulators of animal ecologies through a direct manipulation of animal movement and occurrence, niche dimensions, and vegetation heterogeneity, which, in the process, drive predator–prey relationships, species behavior, and phenotypic trait functions (Whittaker et al., 1973). Also, TRI and elevation control vegetational compositional heterogeneity, where higher and more rugged terrains have more diverse and richer habitats in terms of vegetation resources leading to corresponding higher diversity of mammals (Kerr and Packer, 1997; Yasuhiro et al., 2004; Rahbek et al., 2019b).

Notably, some fundamental caveats must be considered in interpreting and/or applying our findings. Because community ecology studies based on primary sampling assume fair sampling of all resident fauna, imperfect sampling is common, while site conditions such as geographical accessibility and weather conditions can also hinder appropriate sampling. Although the *Cytochrome b* gene provided a well-resolved informative and resolved topology of species associations with deep and terminal divergences well supported by the latest species accounts, congruous with recent observations, these associations must be interpreted within the phylogenetic limitations of *Cytochrome b* for mammalian groups (Tobe et al., 2010). The elevational gradient diversity patterns appear to distinctly vary according to the sampling strategy, i.e., whether the data set covered a single slope or averaged multiple elevational gradient slopes. Future mammal surveys on the Chyulu Hills should employ multi-slope transects to establish with certainty the absence or presence of a mid-elevation richness or diversity peak.

CONCLUSION

We explored the distribution of various phylogenetic and functional diversity indices along elevational gradients of Mt. Kenya and the Chyulu Hills, the two contrasting montane systems, but typical of Afromontane ecosystems. We then tested the associations between the diversity indices and several potential environmental predictors how they relate to the diversity distributions. The association of diversity indices with elevation varied between Mt. Kenya and Chyulu and even between the Mt. Kenya slopes (Chogoria and Sirimon). The phylogenetic and functional richness peaked around mid-elevations in Mt. Kenya, unimodally increased or decreased in Chogoria and Sirimon, and monotonically increased in Chyulu. On the other hand, the divergence and community structure indices were relatively weakly associated with elevation. The tendency of assemblages to be phylogenetically and functionally more closely related than expected by chance decreased with elevation in Mt. Kenya but increased in Chyulu. The annual mean precipitation, temperature seasonality, actual evapotranspiration, and terrain ruggedness were the strongest predictors between the sites and indices. These results provide a holistic understanding of small mammal phylogenetic and functional diversity and assembly patterns, with vital conservation implications. For instance, while Sirimon had fewer clades compared to Chogoria and Chyulu, and even the contiguous Mt. Kenya community (Chogoria and Chyulu), it had the higher phylogenetic and functional richness (PD and FD) and divergence (PD^{MNTD} and FD^{MNTD}), on average. As such, biodiversity prioritization based on species richness alone would neglect the high evolutionary and feature diversity in Sirimon, in the processes, threatening the ecological stability of the Mt. Kenya ecosystem. These slope-wise diversity distributions and associations with abiotic predictors highlight the need for more local-scale habitat level ecological and evolutionary studies, especially in the Afromontane highlands where there has hardly been any progress in local-scale studies based on the phylogenetic and functional diversities.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was reviewed and approved by the Research and Ethics Committee of the Kenya Wildlife Service (permit number: KWS/BRM/5001).

AUTHOR CONTRIBUTIONS

KO, W-YS, X-YL, and X-LJ conceptualized and designed the study. KO, W-YS, X-YL, Z-ZC, QL, SM, EK, and X-LJ conducted fieldwork and contributed to the study design. KO and S-WH performed the lab work. KO wrote the first draft of the manuscript. All authors contributed to consequent revisions of the manuscript and approved the final version for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.742524/full#supplementary-material

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Aboveground Deadwood Biomass and Composition Along Elevation and Land-Use Gradients at Mount Kilimanjaro

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Deadwood is an important structural and functional component of forest ecosystems and biodiversity. As deadwood can make up large portions of the total aboveground biomass, it plays an important role in the terrestrial carbon (C) cycle. Nevertheless, in tropical ecosystems and especially in Africa, quantitative studies on this topic remain scarce. We conducted an aboveground deadwood inventory along two environmental gradients—elevation and land use— at Mt. Kilimanjaro, Tanzania. We used a huge elevation gradient (3690 m) along the southern slope of the mountain to investigate how deadwood is accumulated across different climate and vegetation zones. We also compared habitats that differed from natural forsts in land-use intensity and disturbance history to assess anthropogenic influence on deadwood accumulation. In our inventory we distinguished coarse woody debris (CWD) from fine woody debris (FWD). Furthermore, we calculated the C and nitrogen (N) content of deadwood and how the C/N ratio varied with decomposition stages and elevation. Total amounts of aboveground deadwood ranged from 0.07 ± 0.04 to 73.78 ± 36.26 Mg ha^{-1} (Mean ± 1 SE). Across the elevation gradient, total deadwood accumulation was highest at mid-elevations and reached a near-zero minimum at very low and very high altitudes. This unimodal pattern was mainly driven by the corresponding amount of live aboveground biomass and the combined effects of decomposer communities and climate. Land-use conversion from natural forests into traditional homegardens and commercial plantations, in addition to frequent burning, significantly reduced deadwood biomass, but not past selective logging after 30 years of recovery time. Furthermore, we found that deadwood C content increased with altitude. Our study shows that environmental gradients, especially temperature and precipitation, as well as different anthropogenic disturbances can have considerable effects on both the quantity and composition of deadwood in tropical forests.

Keywords: Tanzania, East African mountains, aboveground deadwood accumulation, decomposition, carbon stock, land-use change, tropical montane forest, tropical mountain

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HIGHLIGHTS

- We conducted an aboveground deadwood inventory across huge elevation and land-use gradients at Mount Kilimanjaro.
- At Kilimanjaro deadwood can account for up to 37.2 % of the total aboveground biomass.
- Deadwood accumulation strongly depends on live wood accumulation.
- Specific land-use histories differentially affect deadwood accumulation.

INTRODUCTION

Deadwood has many important key functions in forest ecosystems. It provides resources and habitats for a wide range of plant and animal groups, including amphibians, reptiles, arthropods, birds, mammals, vascular plants, bryophytes, fungi and lichens, and is therefore an important driver of biodiversity (Spetich et al., 1999). Furthermore, it has the potential to reduce erosion on steep forested slopes and promote soil development (Harmon and Hua, 1991; Bobiec et al., 2005). Through its carbon (C) storage capacity, it can be a considerable atmospheric CO_2 sink (Pan et al., 2011; Iwashita et al., 2013), particularly in the tropics (Martin et al., 2021), and may therefore play an important role in mitigating global change effects. Deadwood currently stores about 8% of the global forest carbon stock (Pan et al., 2011) and 8.5% of atmospheric carbon (Friedlingstein et al., 2019). However, these sinks are offset by losses of C due to deforestation and forest degradation, particularly in tropical regions (Pan et al., 2011; Baccini et al., 2017).

Despite accounting for up to 20 % of the total aboveground biomass, deadwood has often been ignored in forest C budgets (Brown, 2002). Deadwood dynamics have been intensively studied in temperate regions for several decades (Harmon et al., 1986), with a particular focus on how deadwood accumulation is affected by management history (Sippola et al., 1998), stand age and site moisture condition (Spies et al., 1988), and successional stage (Battaglia et al., 1999).However, corresponding research in the tropics is still limited, even though these regions account for ∼33% of the terrestrial net primary production and harbor ∼25% of the global terrestrial C (Bonan, 2008).

Large pieces of standing or fallen dead wood, i.e., coarse woody debris (CWD), have been estimated to store 19–33% of the total aboveground C in tropical forests (Iwashita et al., 2013). However, the scarcity of quantitative data means that drivers of deadwood accumulation in these forests are still poorly understood. Moreover, some of the few existing case studies found contrasting results. For example, Clark et al. (2002) found that the accumulation of CWD was largely unaffected by topographic differences in tropical rain forests in Costa Rica, while Gale (2000) found that topography significantly affected CWD accumulation in Bornean and Ecuadorian lowland rainforests.

Furthermore, fine woody debris (FWD) is often ignored in deadwood inventories because of its presumed small contribution. Nevertheless, Harmon et al. (1995) measured FWD amounts reaching 24.2 Mg ha^{-1} in tropical forests and showed that this biomass pool can be of substantial importance, especially after disturbances like fire or hurricanes.

The speed of deadwood decomposition depends both on biotic factors like the pool of decomposing organisms and on abiotic factors like temperature and soil moisture (Harmon et al., 1986; Molinas-González et al., 2017). Since temperature and moisture schemes change with altitude, we expect that wood decomposition and chemical composition should also vary across an altitudinal gradient (Fravolini et al., 2016).

Until now, few studies have systematically investigated multiple aspects of deadwood along environmental gradients in tropical ecosystems. To improve our understanding of the role that different components of deadwood play in tropical forest ecosystems and the drivers of deadwood dynamics, we investigated deadwood biomass and composition across a large elevational (860–4550 m asl) and land-use gradient on the southern slope of Kilimanjaro, Tanzania. As the world's highest solitary mountain, on a relatively small area (80 × 60 km), Kilimanjaro harbors a wide range of vegetation belts with different habitats and land use types. During the recent past (∼last 30 years), most of these belts have experienced some kind of disturbance – e.g., fire (Hemp, 2005a) and land-use change (Hemp, 2006a) – resulting in different disturbance histories. Both disturbance regimes and climatic conditions (at different elevations) have been identified as important drivers of live aboveground biomass accumulation (Ensslin et al., 2015), as well as vegetation structure (Rutten et al., 2015b) and species composition (Hemp, 2006a,b) and can therefore be expected to also affect deadwood stocks and composition.

We specifically asked:

i) How do aboveground deadwood biomass and composition depend on elevation at Mt. Kilimanjaro and which environmental factors influence these relationships?
ii) Do different land-use regimes and disturbance histories influence deadwood accumulation and composition?
iii) How much C is stored in the deadwood at Kilimanjaro and does this change with elevation?
iv) Does the wood C content of deadwood change with elevation?

MATERIALS AND METHODS

Study Site

Mt. Kilimanjaro is located 300 km south of the equator between 2°45′ and 3°25′ South and 37°00′ and 37°43′ East. It is not only Africa's highest peak but also the highest free-standing mountain in the world. The mountain is an ancient volcano with three peaks (Mawenzi, Shira, Kibo). It covers an elevation gradient from ∼700 m above sea level (asl) to 5895 m asl. This corresponds to a temperature gradient linearly decreasing from 23°C at the lowland savannas to 3°C at the alpine zone and −7°C at the Uhuru-peak (summit) (Hemp, 2006a).

Precipitation at Mt. Kilimanjaro is mainly determined by the biannual passage of the Intertropical Convergence Zone (ITCZ) and is modified by elevation. Due to its equatorial location Mt Kilimanjaro has two distinct annual wet periods – a long rain period from March to May and a short but intense rain period around November (Hemp, 2006a). Precipitation is lowest at the savanna foothills (∼900 mm/year at 800 m asl) and afro-alpine heathlands (∼500 mm/year at 4700 m asl) and peaks at mid-elevation (partly over 3000 mm/year at 2200 m asl; Hemp, 2006a). Mean annual precipitation and temperature data for each of the research plot were provided by Appelhans et al. (2014).

The relatively mild climate in the lower montane zone promotes small holder farming and commercial plantations and farms. This, together with ongoing population growth, has led to the establishment of a variety of natural and anthropogenically altered habitat types at lower and mid elevations (Rutten et al., 2015b; Hemp and Hemp, 2018; **Figure 1**).

Habitat Types at Mt. Kilimanjaro

We used the permanent study plots of the DFG-Research Unit "Kilimanjaro ecosystems under global change: Linking biodiversity, biotic interactions, and biogeochemical ecosystem processes (KiLi)" which cover all main natural and human-modified habitat types across the elevational gradient on Mt. Kilimanjaro[1]. The study plots of the KiLi project cover the 13 most important natural and anthropogenically influenced habitat types on Kilimanjaro (**Figure 1**) occurring in the six main vegetation zones on the southern and eastern slope, where vegetation zonation is stronger and the habitat range wider than on the northern and western slope due to the wetter conditions and the more pronounced climatic and elevational gradient (Hemp, 2006a).

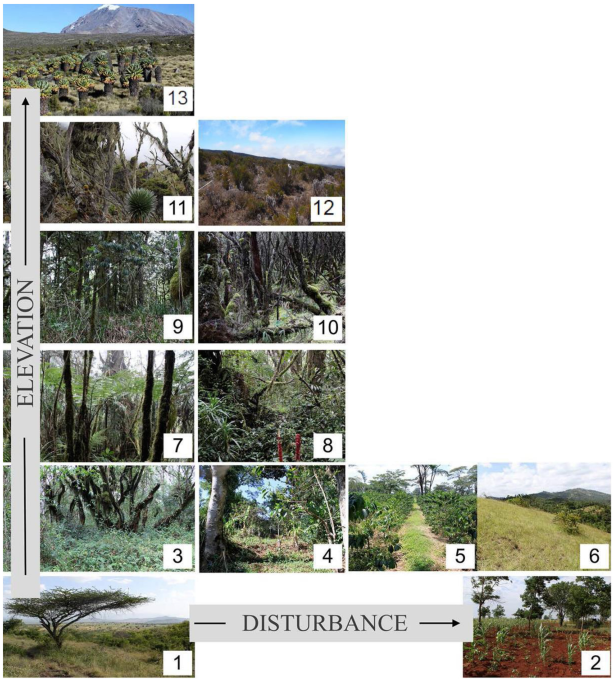

FIGURE 1 | Location of the 13 land-use types along the elevational, and disturbance and land-use gradient. The six land-use types with no or little disturbance are savanna (1), lower montane forest (3), Ocotea forest (7), Podocarpus forest (9), Erica forest (fer), and Helichrysum (hel). The land-use types with medium anthropogenic disturbance are grassland (6), disturbed Ocotea forest (8), disturbed Podocarpus forest (10), and disturbed Erica forest (12). The habitat types with intensive human land-use are maize (2), Chagga homegardens (4), and coffee plantations (5).

Six of these habitats are in a natural and undisturbed stage and seven are characterized by different land-use intensities (LUI) or disturbance histories (**Supplementary Table 3**). The lowest habitats on the foothills in the colline savannah belt (800–1100 m asl) are dominated by crop production (maize, beans, and sunflowers) and only a few savannah areas are left (Hemp, 2006a). From 1100 to 2000 m asl, the zone characterized by the highest human population density at the mountain (Hemp, 2006a), the most widespread land use types is the traditional agroforestry system of the local Chagga people ("Chagga homegardens"). Bananas, beans and coffee are cultivated alongside some interspersed forest trees for shade provision. Furthermore, in this zone, the Chagga people have turned forests into frequently cut grasslands for fodder collection. Commercial coffee farms have also been established at this elevation belt. Relicts of natural forests are mostly restricted to steep valleys and gorges harboring the tallest trees in Africa (Hemp, 2006a; Hemp et al., 2017). Above the densely populated submontane zone begins the montane forest belt (1800 to 2800 m asl). The lower part is characterized by *Ocotea usambarensis* dominated forests, which were partly subjected to selective logging until 1984 (Agrawala et al., 2003; Hemp, 2006a). Several sawmills operating on the southern slopes of the mountain removed precious large timber trees, mainly *Ocotea usambarens*. Pit-sawyers and saw millers harvested 1,876,321 m^3 of timber from various indigenous trees on the whole mountain between 1965 and 1984. Since then, only scattered illegal logging has occurred in some remote areas. These illegal activities decreased after the incorporation of the forest belt into the national park in 2006. In the upper areas of Kilimanjaro fires are frequent and mainly lit by people (honey collectors and poachers). Most of these fires occur at the end of the dry seasons and stop at the beginning of the rainy seasons. During the last decades, about 150 km^2 of upper montane and subalpine forests were burnt (Hemp, 2005b). The upper montane zone (2800 to 3200 m asl) is dominated by *Podocarpus latifolius* forests. Large parts of these forest were destroyed by fire and then replaced by *Erica excelsa* forests creating a mosaic of burnt and unburnt areas (Hemp and Beck, 2001; Hemp, 2006a). In the subalpine zone (3200 to 4000 m asl) most of the former *Erica trimera* forests (Africa's highest forests) have been degraded after fire in the *ericaceous* shrublands (Hemp, 2006a,b). The last and highest zone is characterized by alpine vegetation (4000 to 4600 m asl) and contains mainly *Helichrysum* cushion plants and tussock grasses (Hemp, 2006a).

In this study, each habitat type is represented by five replicate plots, with the exception of coffee plantations (6 replicates) and alpine vegetation (4 replicates), distributed

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[1] https://www.kilimanjaro.biozentrum.uni-wuerzburg.de/

along an east-west and an elevational gradient within each vegetation zone. The 65 plots, each of 0.25 ha, were distributed over five elevational transects on the southern slope of Mt. Kilimanjaro, covering an elevational gradient from 870 to 4550 m a.s.l. Minimum distance between transects was 12.2 km on the foothills and 4.6 km in the alpine zone, and minimum distance between plots was approximately 300 m. For each site, plot position (latitude, longitude and elevation) was recorded with GPS. For more details see e.g., Rutten et al. (2015b). Moreover, for each plot, mean annual temperature was obtained from in-situ measures by temperature loggers (data logger DK320, Driesen and Kern GmbH, Bad Bramstedt, Germany) and mean annual precipitation was modeled using long-term observations based on a 15-year dataset from a network of rain gauges distributed across the whole mountain (Hemp, 2006a; Appelhans et al., 2014).

Sampling

Within the center of each 50 × 50 m KiLi study plot, an area of 50 × 20 m (1000 m^2) was surveyed for aboveground deadwood during spring and autumn 2015. All pieces with a diameter > 10 cm at their larger end were classified as coarse woody debris (CWD). In this class, pieces were further described as standing dead trees (snags), downed trees (logs) or stumps (standing dead trees not exceeding 1.5 m in height). For snags, diameter at breast height (DBH) and top diameter were measured using a 100 cm caliper or a measuring tape. Whenever the top diameter was not possible to reach, it was visually estimated. The height of snags was measured with an ultra-sonic hypsometer (Vertex IV Hypsometer, Haglöf, Langsale, Sweden). For logs and stumps, three diameters were measured at the big, middle and small ends using the 100 cm caliper. For all pieces that clearly deviated from a circular circumference, two different diameters perpendicular to each other were measured. The length of each piece was measured using a measure tape. Furthermore, the volume of hollow parts in CWD pieces was assessed as described below. Each CWD piece was then ascribed to one of five decomposition classes according to the following criteria after Baker et al. (2007):

(1) Solid, recently fallen wood with intact bark and fine branches attached. (2) Solid wood without fine branches, bark starting to fall off. (3) Non-solid wood, but still difficult to push a knife into. (4) Soft rotten wood, easy to push a knife into. (5) Soft rotten wood, collapsing easily when standing upon it.

Finally, wood samples of CWD pieces for subsequent C/N analysis were collected using an increment corer (Increment borer 60cm, Suunto, Vantaa, Finland), a handsaw or a machete such as to include a representative wood transect from the core to the bark. Whenever possible, at least 10 wood pieces (logs, stumps or snags) per plot were randomly chosen. When it was impossible to collect 10 samples, the maximum available number was taken.

Fine woody debris (FWD) consisted of all pieces with a diameter < 10 cm at the bigger end. To record FWD, 10 subplots, each consisting of $1m^2$, were established randomly within the original plot and within these subplots, all FWD was collected and weighed directly in the field using a spring balance.

We excluded banana plants in the entire inventory since the focus was only on woody plants. Even though some habitats are densely covered with bananas, they represent an ephemeral biomass pool, since the debris of these plants is normally immediately gathered by local people.

Wood Density

To estimate wood density of CWD samples, the volume of 195 wood samples from those taken for C/N analyses was measured to the nearest 0.25 ml using the volumetric water displacement method (Ravindranath and Ostwald, 2008). The samples were then oven dried to constant weight (at 80° for at least 72 h) and weighed to the nearest g. Finally, the wood density of each sample was calculated as:

$$\rho = \frac{dry\ weight\ [kg]}{fresh\ volume\ [m^{-3}]} \tag{1}$$

Since we did not distinguish between different tree species, we averaged the density at a plot level and used this approximation for further calculations (**Supplementary Table 4**).

Coarse Woody Debris Volume and Biomass Calculation

To calculate CWD volume, we used equations proposed by Harmon and Sexton (1996). First, we created a round equivalent for all wood pieces that deviated strongly from a circular circumference using the equations [2 & 3]:

$$A = D_{max} \times D_{min} \tag{2}$$

$$D_{round} = \sqrt{A} \tag{3}$$

where, D_{max},D_{min}, and D_{round} are the maximum (for standardization, diameter of standing deadwood was measured at breast height; DBH), minimum and round equivalent diameters [m], respectively, A[m^2] is the area.

The volume of logs and stumps was calculated with Newton's formula:

$$V = L \times \frac{A_S + 4A_M + A_B}{6} \tag{4}$$

where, V = Volume [m^3], A_S, A_M and A_B = Area [m^2] at small end, middle and big end, respectively, (calculated with corresponding diameter measurements), and L = Length of the piece [m].

For snags lacking a D_{min} measurement or estimate, we used the slope of the regression line between D_{max} and D_{min} of all pieces, for which both measurements could be obtained, to estimate D_{min}. Then we calculated the volume using the formula for the frustum of a paraboloid:

$$V = L \times \frac{A_S + A_B}{2} \tag{5}$$

where, V = Volume [m^3], A_S and A_B = Area at small and big end, respectively [m^2], and L = Length of the piece [m]. The same equations were used to calculate the volume of holes of hollow pieces, which then was subtracted from the total volume to get the actual wood volume per piece.

Biomass of a CWD piece was calculated as:

$$M = \rho \times V \tag{6}$$

where, M = mass of wood piece [t], ρ = wood density [t/m^3] (averaged by plot), V = volume of the wood piece [m^3]. Finally, the sum of CWD was calculated at the plot level.

For one natural plot (FPO5) samples were lost. Therefore, we averaged the density of all other plots of the same habitat type and used this measure for the biomass calculation.

C/N Analysis

A total of 274 CWD samples were thoroughly ground using a mixer mill (Retsch MM400) and then concentrations of C and Nitrogen (N) were determined using a C/N analyser (vario EL cube, Elementar) at the Institute of Geography, University of Bern, Switzerland. Mean C and N content per plot were calculated as the arithmetical average of C and N content of analyzed wood samples from respective research plots.

Wood C Pools

We multiplied the average wood C content of each plot by its corresponding deadwood biomass values to estimate C pools. For plots where it was not possible to get wood C content measurements due to the absence of deadwood (see **Supplementary Table 4**), we used the mean C content of all plots of the same habitat type or elevation belt as an approximation.

Statistical Analysis

We applied linear regression models to find out whether wood density and C content depended on elevation. To test for differences in these two measurements between different habitat types (section "Habitat Types at Mt. Kilimanjaro"), we performed Student's *t*-tests and Tukey's HSD tests.

In order to analyse how deadwood biomass accumulation and the corresponding C pools varied with elevation and precipitation, we focused exclusively on natural, undisturbed plots (see **Figure 1**). We used linear regression models with total deadwood biomass (TDW), CWD or FWD and the corresponding deadwood C pools (CWD C, FWD C, and TWD C) as response variables and elevation or precipitation as explanatory variables. We fitted linear, quadratic and cubic functions to the data and chose the best model according to the Akaike Information Criterion (AIC). Whenever the normality assumption of the residuals was violated, the response variable was log-transformed prior to the analysis. In cases where log-transformation of the data did not result in normally distributed residuals, we used additional statistical techniques (generalized linear models (glm), Kruskal-Wallis rank sum test, ANOVA test with log- transformed data) to check the robustness of the results. If all tests led to similar results, we assumed that they were robust despite not meeting the normality assumption.

To analyse differences in deadwood biomass content between natural and disturbed plots (section "Habitat Types at Mt. Kilimanjaro"), we used Student's *t*-tests and Tukey's HSD tests.

All analyses were performed using RStudio Version 0.99.489 (RStudio Team, 2020).

RESULTS

Wood Properties Across Elevation and Decomposition Stages

In total we measured the diameters and lengths of 700 CWD pieces (500 logs, 106 snags and 94 stumps) and calculated the aboveground deadwood volume based on these measurements. Mean wood density per plot, derived from 195 samples, was 0.34 ± 0.01 Mg m^{-3} and did not vary significantly between habitat types or different elevations (**Supplementary Table 4**). However, wood density decreased with increasing decomposition stage ($R^2 = 0.10$, $F_{4,189} = 6.43$, $P < 0.01$: **Table 1**). The chemical composition (C and N content) of wood samples was measured for 274 CWD samples and varied across different decomposition classes (**Table 1**) as well as elevation (**Figure 2**). Percentage of both C and N in CWD samples increased with increasing decomposition (**Table 1**: $R^2 = 0.03$, $F_{4,269} = 3.01$, $P < 0.01$, and $R^2 = 0.04$, $F_{4,269} = 4.21$, $P < 0.01$, respectively). The C/N ratio decreased with increasing decomposition stage ($R^2 = 0.05$, $F_{4,269} = 4.54$, $P < 0.01$). The percentage of C in CWD samples increased significantly with elevation ($R^2 = 0.26$, $F_{1,32} = 12.68$, $P < 0.01$; **Figure 2**), while % N remained constant.

Deadwood Biomass and C Pools in Natural Plots Over Elevation and Precipitation

All three deadwood pools (FWD, CWD, and TDW) showed similar unimodal distributions along elevation (**Figure 3**). Even though we found an increase in C content of deadwood with altitude, this did not change the general relationship between deadwood biomass and deadwood C pool, because the absolute increase of C content was only 4%, while biomass difference was almost 500 times. Therefore, the distributions of CWD C and FWD C along the elevation gradient strongly followed the ones of CWD and FWD. For simplicity, we only discuss CWD and FWD and provide information on the corresponding C pools in **Table 2** and **Supplementary Figures 1, 2**.

The maximum amounts of CWD and CWD C (87.46 Mg ha^{-1} and 42.42 Mg C ha^{-1}) were measured in an *Ocotea* forest plot (2540 m asl) and decreased to zero in both the lowland savanna and the alpine *Helichrysum* zones (CWD; $R^2 = 0.6304$, $F_{2,27} = 25.73$, $P < 0.001$, CWD C; $R^2 = 0.58$, $F_{2,27} = 20.9$, $P < 0.001$). The lowest and the highest zones with more than one plot containing any CWD were the lower montane and subalpine zones, respectively.

The FWD and FWD C also peaked in an *Ocotea* forest (25.57 Mg ha^{-1} and 12.33 Mg C ha^{-1} at 2260 m asl) and decreased to minimal amounts in the savanna zone (0.09 Mg ha^{-1} and 0.05 Mg C ha^{-1}, respectively, at 1130 m asl) and in the *Helichrysum* zone (0.17 Mg ha^{-1}/0.08 Mg C ha^{-1}, respectively, at 4240 m asl) (FWD; $R^2 = 0.60$, $F_{2,27} = 22.8$, $P < 0.001$, FWD C; $R^2 = 0.55$, $F_{2,27} = 19.19$, $P < 0.001$).

TABLE 1 | Decomposition classes of CWD samples and corresponding wood densities.

dc	wd (Mg m^{-3})	% C	% N	C/N ratio	n.wd	n.C/N
1	0.44 (0.02)	47.7 (0.51)	0.297 (0.037)	251.0 (23.3)	14	19
2	0.40 (0.01)	48.0 (0.14)	0.270 (0.015)	251.7 (9.5)	29	62
3	0.37 (0.01)	48.6 (0.16)	0.279 (0.010)	228.6 (7.3)	88	113
4	0.28 (0.02)	48.5 (0.26)	0.339 (0.025)	195.8 (11.1)	30	42
5	0.27 (0.01)	49.6 (0.27)	0.418 (0.024)	158.2 (9.1)	29	38

Wood density (wd), %C, %N, and C/N ratio were averaged for each decomposition class (dc). SE in parentheses. n.wd and n.C/N refer to the number of density and C/N samples, respectively, for each decomposition class. Decomposition classes are: (1) Solid, recently fallen wood with intact bark and fine branches attached, (2) Solid wood without fine branches, bark starting to fall off, (3) Non-solid wood, but still difficult to push a knife into it, (4) Soft rotten wood, easy to push a knife into it, and (5) Soft rotten wood, collapsing easily when standing upon it (Baker et al., 2007).

TWD and TWD C were highest in a montane forest plot (105.36 Mg ha^{-1}/51.11 Mg C ha^{-1}, respectively, at 2540 m asl) and decreased to minimal amounts in the savanna zone (0.10 Mg ha^{-1}/0.04 Mg C ha^{-1}, respectively, at 1130 m asl) and in the *Helichrysum* zone (0.17 Mg ha^{-1} 0.08 Mg C ha^{-1} respectively at 4240 m asl; TWD; $R^2 = 0.74$, $F_{2,27} = 41.4$, $P < 0.001$, TWD C; $R^2 = 0.70$, $F_{2,27} = 35.9$, $P < 0.001$).

Consequently, total aboveground biomass and C pool (TAGB and TAGB C, respectively; calculated as the live aboveground biomass or C pool from Ensslin et al. (2015) plus TWD or TWD C, respectively) peaked in a lower montane forest plot (699.10 Mg ha^{-1}/ 336.73 Mg C ha^{-1}, respectively, at 1650 m asl), with the low and high elevation minima measured in a savanna plot (4.00 Mg ha^{-1}/1.95 Mg C ha^{-1}, respectively, at 1130 m asl) and a *Helichrysum* plot (1.47 Mg ha^{-1}/0.68 Mg C ha^{-1}, respectively at 4240 m asl) (TAGB; $R^2 = 0.63$, $F_{2,27} = 25.73$, $P < 0.001$, TAGB C; $R^2 = 0.89$, $F_{2,20} = 92.2$, $P < 0.001$).

The CWD, CWD C, FWD, TWD, and TWD C significantly increased with increasing mean annual precipitation (MAP) (CWD: $R^2 = 0.60$, $F_{1,28} = 56.5$, $P < 0.001$; CWD C: $R^2 = 0.61$, $F_{1,28} = 45.93$, $P < 0.001$ FWD: $R^2 = 0.59$, $F_{1,28} = 44.08$, $P < 0.01$; and TWD: $R^2 = 0.71$, $F_{1,28} = 73.41$, $P < 0.001$, TWD C: $R^2 = 0.78$, $F_{1,21} = 80.18$, $P < 0.001$ **Figure 4**).

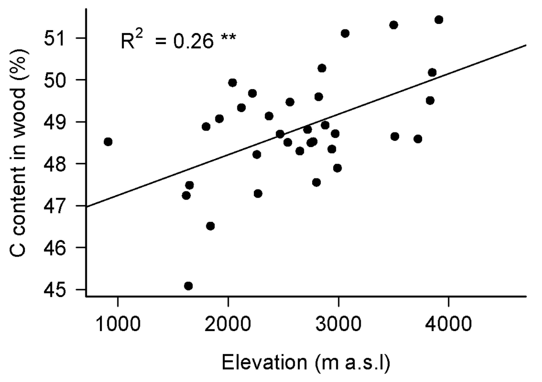

FIGURE 2 | C content in CWD samples as a function of elevation. The percentage of C in CWD, averaged for each plot, significantly increased with increasing elevation ($R^2 = 0.26$, $F_{1,32} = 12.68$, $P < 0.01$).

We did not find any significant relationship between deadwood composition (debris type and decomposition stage; **Supplementary Tables 1, 2**) and altitude or precipitation.

Impacts of Land Use and Disturbance History on Deadwood Biomass and C Pools

Significant differences between natural and disturbed plots with regard to deadwood biomass and C pools were found in the lower montane and the alpine zone. In the lower montane zone mean CWD and CWD C was more than 7-fold higher in natural forests (17.47 Mg ha^{-1}/8.43 Mg C ha^{-1}, respectively) than in homegardens (3.93 Mg ha^{-1}/1.04 Mg C ha^{-1}, respectively; CWD; $P < 0.01$, CWD C; $P < 0.01$), whereas grasslands and coffee plantations did not harbor any CWD and CWD C at all (**Figure 5A** and **Supplementary Figure 2A**). In the same zone, mean FWD/ FWD C of natural forests (5.65 Mg ha^{-1}/2.74 Mg C ha^{-1}, respectively) more than doubled compared with disturbed plots (homegardens = 1.91 Mg ha^{-1}/0.87 Mg C ha^{-1}, grasslands = 0.07 Mg ha^{-1}/0.04 Mg C ha^{-1}, coffee plantations = 0.58 Mg ha^{-1}/0.28 Mg C ha^{-1}, respectively; **Figure 5B** and **Supplementary Figure 2B**). In the subalpine zone, though low in values, natural *Erica* forests harbored

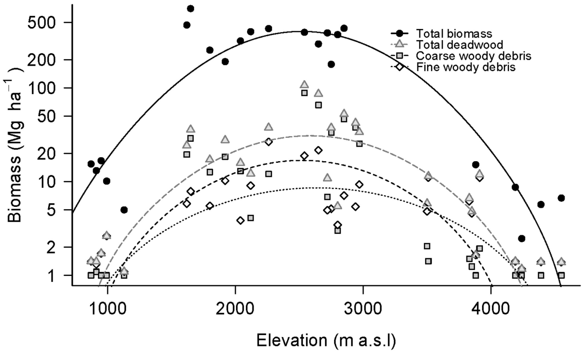

FIGURE 3 | Aboveground biomass over the elevation gradient at Mt. Kilimanjaro. Total biomass was calculated as live aboveground woody biomass [Ensslin et al. (2015)] + TWD. Quadratic functions were fitted to total biomass ($R^2 = 0.83$, $F_{2,20} = 55.03$, $P < 0.001$), TWD ($R^2 = 0.74$, $F_{2,27} = 41.4$, $P < 0.001$), CWD ($R^2 = 0.6304$, $F_{2,27} = 25.73$, $P < 0.001$), and FWD ($R^2 = 0.60$, $F_{2,27} = 22.8$, $P < 0.01$). Please note the log-scale.

TABLE 2 | Distribution of deadwood components and deadwood C pool in different habitat types.

Zone	Type		CWD	FWD	TWD	Mg C ha^{-1}
Savanna	**Savanna**	Mean	0.02 (0.02)	0.63 (0.27)	0.65 (0.26)	0.31 (0.13)
		Range	0.0–0.1	0.1–1.6	0.1–1.6	0.0–0.8
		%	0.0–0.8	2.4–17.9	2.4–17.9	2.3–14.3
	Maize field	Mean	0 (0.00)	0.04 (0.02)	0.04 (0.02)	0.02 (0.01)
		Range	0.0–0.0	0.0–0.1	0.0–0.1	0.0–0.1
		%	0.0–0.0	0.0–0.6	0.0–0.6	0.0–0.6
Lower montane	**Natural forest**	Mean	17.47 (2.69)	5.65 (1.09)	23.12 (3.65)	11.17 (1.69)
		Range	11.6–28.0	2.9–9.2	14.8–34.8	7.4–16.5
		%	3.8–9.2	0.9–4.9	4.7–14.0	4.9–14.3
	Home garden	Mean	3.93 (1.58)	1.91 (0.16)	5.94 (1.63)	1.91 (0.64)
		Range	0.1–7.5	1.5–2.3	1.7–9.7	0.8–4.4
		%	0.2–5.3	1.3–3.5	2.8–6.9	2.5–6.5
	Grassland	Mean	0 (0.00)	0.07 (0.04)	0.53 (0.14)	0.04 (0.02)
		Range	0.0–0.0	0.0–0.3	0.0–0.3	0.0–0.1
		%	0.0–0.0	0.0–6.8	0.0–6.8	0.0–6.6
	Coffee plantation	Mean	0 (0.00)	0.58 (0.14)	0.07 (0.04)	0.28 (0.06)
		Range	0.0–0.0	0.1–1.0	0.1–1.0	0.0–0.5
		%	0.0–0.0	0.4–2.2	0.4–2.2	0.3–2.2
Montane	***Ocotea* forest**	Mean	39.71 (15.99)	15.25 (3.99)	54.96 (17.39)	26.62 (8.41)
		Range	3.1–87.5	4.1–25.6	11.1–105.4	5.5–51.1
		%	0.8–22.4	2.0–7.0	2.8–29.1	2.9–29.1
	Logged	Mean	67.68 (36.16)	6.10 (1.59)	73.78 (36.26)	40.92 (17.59)
		Range	8.4–206.7	2.5–11.3	12.3–211.4	6.1–100.0
		%	2.3–36.4	0.6–3.2	3.3–37.2	3.4–36.8
Upper montane	***Podocarpus* forest**	Mean	28.12 (7.69)	5.04 (1.00)	33.81 (7.95)	13.73 (4.51)
		Range	2.0–45.5	2.5–8.3	4.4–51.6	2.1–26.0
		%	0.5–10.6	0.7–1.4	1.2–12.0	1.2–12.4
	Burned	Mean	3.36 (1.27)	4.76 (0.52)	8.12 (1.28)	5.23 (1.56)
		Range	0.6–17.3	3.8–6.7	5.5–21.6	2.7–11.0
		%	NA	NA	NA	NA
Subalpine	***Erica* forest**	Mean	0.63 (0.15)	6.51 (1.45)	7.14 (1.48)	3.58 (0.74)
		Range	0.2–1.0	3.6–10.0	3.8–10.9	1.9–5.6
		%	NA	NA	NA	NA
	Erica disturbed	Mean	0.06 (0.06)	2.24 (0.60)	2.3 (0.63)	1.11 (0.30)
		Range	0.0–0.3	0.6–4.0	0.6–4.0	0.3–1.9
		%	NA	NA	NA	NA
Alpine	***Helichrysum* zone**	Mean	0 (0.00)	0.4 (0.08)	0.4 (0.08)	0.19 (0.04)
		Range	0.0–0.0	0.2–0.7	0.2–0.7	0.1–0.3
		%	0.0–0.0	4.6–11.6	4.6–11.6	4.6–12.0

The first lines refer to the mean (± SE, in parentheses) amount (Mg ha^{-1}) of each deadwood component and mean total deadwood C pool (Mg C ha^{-1}) in the corresponding habitat type. Second lines refer to the corresponding ranges (Mg ha^{-1} or Mg C ha^{-1}). Third lines express the range of deadwood components as percentage of total aboveground biomass and the deadwood C pool as percentage of total aboveground C pool.

Deadwood biomass is given for coarse woody debris (CWD), fine woody debris (FWD), and as total deadwood biomass (TWD). Total carbon refers to the amount of C in the TWD biomass. Natural habitats in bold letters.

significantly more CWD and CWD C (0.63 Mg ha^{-1} /0.32 Mg C ha^{-1}, respectively) than burned Erica forests (0.06 Mg ha^{-1}/0.03 Mg C ha^{-1}, respectively; CWD; $P = 0.02$, CWD C; $P = 0.02$ **Figure 5A** and **Supplementary Figure 2A**). Mean FWD and FWD C in natural *Erica* forests (6.51 Mg ha^{-1} and 3.26 Mg C ha^{-1}, respectively) were almost three times larger than in burned plots (2.24 Mg ha^{-1} and 1.08 Mg C ha^{-1}, respectively) (FWD; $P = 0.04$; FWD C; $P = 0.04$, **Figure 5B** and **Supplementary Figure 2B**).

Wood density, C and N content and C/N-Ratio did not differ between CWD samples from natural and disturbed plots.

Differences with regard to deadwood composition were only significant in the lower montane zone. Natural forests harbored more log biomass than homegardens ($P < 0.01$), coffee plantations ($P < 0.01$) and grasslands ($P < 0.01$), and more stump biomass than coffee plantations ($P = 0.03$) and grasslands ($P = 0.04$). Furthermore, natural forests also harbored more wood of decomposition class 3 than coffee plantations ($P < 0.01$) and grasslands ($P = 0.01$) and more wood of decomposition class 4 than coffee plantations ($P = 0.02$), grasslands ($P = 0.03$) and homegardens ($P = 0.04$) (**Supplementary Table 2**).

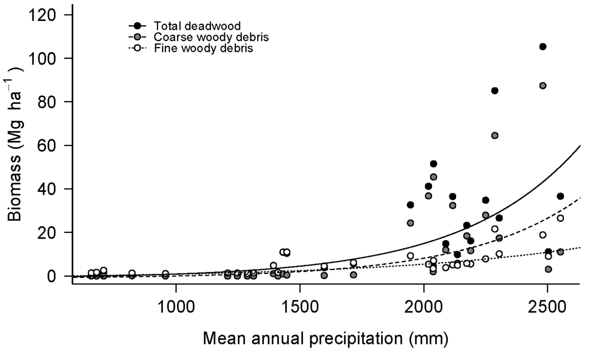

FIGURE 4 | Aboveground biomass as a function of precipitation. Linear functions were fitted to log-transformed biomass data. TWD (R^2 = 0.71, $F_{1,28}$ = 73.41, $P < 0.01$), CWD (R^2 = 0.60, $F_{1,28}$ = 56.5, $P < 0.01$), and FWD (R^2 = 0.59, $F_{1,28}$ = 44.08, $P < 0.01$).

DISCUSSION

Wood Properties Across Elevation and Decomposition Stages

Martin et al. (2021) calculated an average dead wood C content of 47.16% (ranging from 38.66 to 55.66%) for tropical trees globally. This value is lower than what we found on Kilimanjaro (48.57%, ranging from 40.67 to 51.44%). Many studies investigating the C pool of deadwood used such average generalized values for wood C content (e.g., Delaney et al., 1997; Davis et al., 2003; Sierra et al., 2007). In our study, we showed that the C content in CWD samples along the elevation gradient of Kilimanjaro significantly increased (**Figure 2**), although this increase in C was also affected by changes in wood densities of different decomposition classes (see also Merganičová and Merganič, 2010). Since temperature decreases linerarly with increasing altitude, tropical natural elevation gradients may be used as real-world laboratories to assess the impact of rising mean annual temperature (MAT) on the function and structure of tropical forests (Malhi et al., 2010). Our results indicate that climatic factors of the wood sample's origin, such as temperature, may influence its C content suggesting that deadwood C content decreases with increasing MAT. Hishinuma et al. (2015) conducted a detailed analysis of the chemical composition of CWD originating from sites with different climatic conditions. They found that samples from a subalpine environment had a higher lignin content than samples from subtropical environments, while cellulose and hemicellulose contents did not differ strongly (Hishinuma et al., 2015). This may support our findings that deadwood in higher or colder environments contains a higher proportion of C than deadwood from warmer or lower environments, since C is a major compound of lignin. Slower growth and the need to build more resistant tissues (with higher lignin content) in harsh and cold environments may be a possible explanation for these findings. However, a study from the Swiss Alps did not reveal any change in deadwood C content along an elevation gradient of 800 m (corresponding to a ∼6.2°C change; Petrillo et al., 2016).

Other studies showed that species-specific wood C content can vary substantially among tropical trees (Martin and Thomas, 2011) and that taxonomic division also explained a significant proportion of the variability in deadwood C content, with gymnosperm deadwood have 2.0% higher C content on average than angiosperms (Martin et al., 2021). Since the gymnosperm *Podocarpus latifolius* and *Erica* species (*E. excelsa* and *trimera*), with their dense lignin-rich wood, start to prevail from the upper montane zone upward and become monodominant at the highest elevations (Hemp, 2006b), this could also explain part of the increase in C content with elevation.

Another possible explanation of this elevational gradient in C content could be the import of soil particles and soluble nutrients into dead wood by soil macrofauna – in particular, termites (Buxton, 1981)—which could reduce dead wood C content through the decomposition process (Martin et al., 2021), primarily in the lower elevations of Kilimanjaro, where termites are widespread.

Wood density decreased by almost 50 % across the decomposition stages (**Table 1**). This decrease can be explained by the increasing loss of wood structure and has been reported by other studies from both tropical and temperate regions (Iwashita et al., 2013; Petrillo et al., 2016). The strong decrease in wood density we found in this study highlights the importance of considering such changes when calculating C pools, since ignoring them could lead to substantial over- or underestimations of C pools (see also Merganičová and Merganič, 2010; Harmon et al., 2011).

Even though the percentages of both C and N in wood samples increased with decomposition stage, the corresponding C/N ratio decreased although the absolute increment of C content across decomposition was much stronger than the one of N (**Table 1**). Studies of several forest types also reported increases in deadwood N content with decomposition (Bütler et al., 2007; Iwashita et al., 2013; Petrillo et al., 2016). Increased biological activity could be a plausible explanation. As decay progresses, wood becomes increasingly accessible to different groups of decomposers, such as bacteria, plants and fungi. During wood decay, fungi may retain the N of the original substrate while wood mass decreases (Lambert et al., 1980). At the same time groups of bacteria may fix atmospheric N, while plants and fungi translocate N from the soil to the wood (Sollins et al., 1987; Bütler et al., 2007). In a global study, Martin et al. (2021) found a similar increase in C content with increasing decomposition. Cellulose and hemicellulose generally decompose more rapidly than lignin (Harmon et al., 2013), and lignin has a considerably higher C concentration than cellulose/hemicellulose (Pettersen, 1984); thus, the increase of C content through decomposition may be a function of increasing lignin concentrations (Martin et al., 2021).

Deadwood Biomass and Carbon in Natural Plots Over Elevation and Precipitation

The distributions of both CWD and FWD at Mt. Kilimanjaro were similar to those of live aboveground biomass (Ensslin et al., 2015; **Figure 3**). Even though CWD and FWD are positively

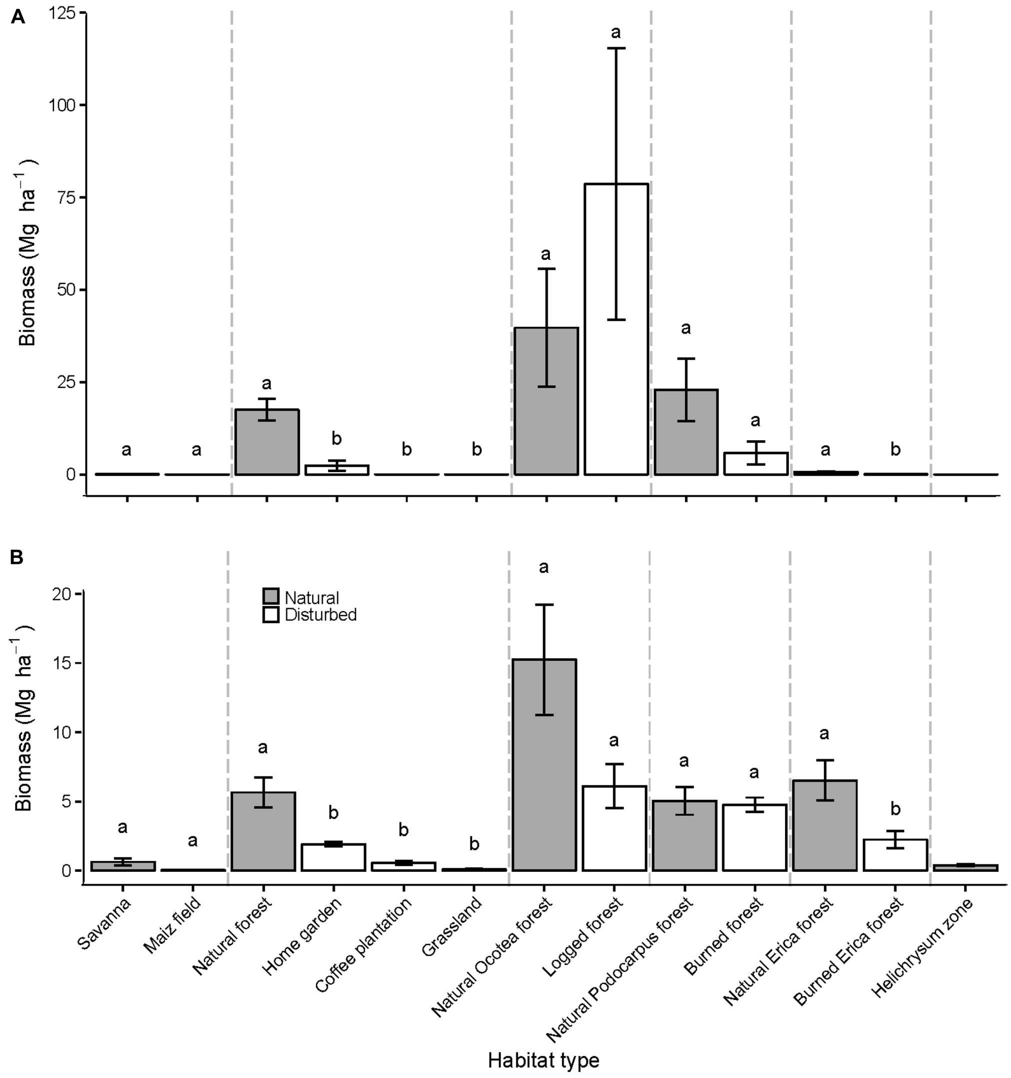

FIGURE 5 | Aboveground deadwood biomass in 13 different habitat types. Bars indicate mean ± SE CWD **(A)** and FWD **(B)** biomass per plot type (each based on five replicate plots, except for coffee plantations which were represented with six plots). Gray bars indicate natural habitat types; white bars indicate plots that experienced some kind of disturbance. Dashed gray lines separate different elevation zones (savanna, lower montane, montane, upper montane, and subalpine alpine zones from left to right). Bars not sharing a letter are significantly different from each other at the $P < 0.05$ level.

related to MAP (**Figure 4**), the causation behind this relationship is probably to some degree indirect. Ensslin et al. (2015) showed that live aboveground biomass was strongly related to MAP ($R^2 = 0.85^{***}$). The correlations between CWD and MAP as well as FWD and MAP were much weaker, however, ($R^2 = 0.60^{***}$ and 0.71^{***}, respectively). This indicates that higher precipitation promotes deadwood accumulation at least partly due to increased productivity. Another factor, also related to precipitation, is enhanced tree fall and breaking of branches after heavy rains, when the dense epiphyte loads in the humid montane *Ocotea* forests become too heavy (Hemp, 2006b).

Dead wood dynamics are governed not only by dead wood accumulation but also by decomposition. Wood decomposition rates were shown to be highly climate-sensitive (Chambers et al., 2000; Bradford et al., 2014; Lustenhouwer et al., 2020), being highest in the tropics (Seibold et al., 2021), and driven by the complex interplay between temperature and precipitation with the decomposer community (Seibold et al., 2021). The most important biotic agents of wood decomposition are microbes (i.e., fungi and bacteria) and insects such as termites and beetles (Bradford et al., 2014; Ulyshen, 2016; Lustenhouwer et al., 2020), but the role of insects varies strongly across the globe (Seibold et al., 2021) as important decomposer groups (e.g., termites) are limited in their geographical distribution (Bradford et al., 2014). At Kilimanjaro termites occur only in the foothills. At a global-scale, decomposition rates and the net effect of insects on decomposition increased with increasing temperature (Seibold et al., 2021). Similar positive relationships with temperature have been reported for the decomposition of non-woody litter (Wall et al., 2008). Using the same research plots on Kilimanjaro that we did, Shen et al. (2020) found that soil bacterial diversity had a U-shaped pattern (mainly influenced by pH) while soil fungal diversity monotonically decreased following the temperature gradient. Two plots of our study (SAV4 and FLM3) were part of the above-mentioned global study of Seibold et al. (2021). Decomposition in the savanna, where termites played an important role and mean annual temperature was 23.9°C, was higher with an annual mass loss (i.e., decomposition rate) of 21%t, whereas decomposition rate in the lower montane forest with a mean annual temperature of 16.5°C was 16%. In summary, these combined effects of climate and decomposer communities could be another reason for the observed hump-shaped pattern in deadwood stock along the elevation gradient of Kilimanjaro with maxima in mid-elevations.

In savanna woodlands, CWD was mostly absent even from natural plots. Besides the high decomposition rate, the reason for this could be that grasses and shrubs dominate this zone and trees with a diameter > 10 cm are scarce. Frequent fire occurrence and grazing by large mammals have been shown to favor the expansion of grasses at the expense of woody species in East African savannas (Gurevitch et al., 2006). Hence, the amount of live biomass is not very high and is mainly comprised of shrubs and non-woody plants, which were beyond the scope of this study. However, local people enter the plots to collect deadwood as firewood. Therefore, the deadwood quantities for natural savanna plots without any human influence could be higher than in this study.

The quantity of total deadwood we observed in the lower montane forests was only about half of the total amount observed in undisturbed tropical lower montane moist forests in Venezuela (Delaney et al., 1997), but similar to the mean amount of deadwood in tropical premontane moist primary forests in Colombia (Sierra et al., 2007). The corresponding live tree biomass quantities were similar in plots of the Venezuelan and our study sites, but considerably lower at the Colombian plots. As in the savanna, deadwood might have been underestimated in this zone, due to its accessible location near local Chagga settlements. Even though this zone belongs to the national park, local people are allowed to collect firewood for cooking. However, they are not allowed to fell live trees, which probably leads to a discrepancy between the real natural stage of live and deadwood components in this zone.

In the montane zone, natural forests harbored considerably more CWD and FWD than the lower montane zone. Here, natural forests contained the highest amount of deadwood across the elevation gradient of Mt. Kilimanjaro. Huge trees like *O. usambarensis* grow in this zone and provide large amounts of biomass. The largest *O. usamberensis* snag in a plot of the montane zone was estimated to harbor ∼17 Mg of deadwood. However, such huge individuals were sparsely scattered and not present in all plots of the montane zone, which partly explains the large variability in CWD content among the plots of this zone (SD of 82.3 Mg ha^{-1}). Nevertheless, compared with the total amount of deadwood of undisturbed tropical montane wet forests in Venezuela (Delaney et al., 1997), deadwood (CWD and FWD) appears to be a much more important component at Mt. Kilimanjaro (1.4–5.9% vs. 2.8–29.1% of total aboveground woody biomass, respectively).

In the upper montane zone, both CWD and FWD declined again. To our knowledge there are no other studies that investigated deadwood in similar climatic zones (upper montane wet tropical forest). However, compared to the range of CWD C in other tropical moist forests [14.7–25.4 Mg C ha^{-1}; (Clark et al., 2002; Raich et al., 2006; Iwashita et al., 2013)], the CWD C pool of this zone is rather low.

In the subalpine zone, where 10–15 m high *Erica trimera* individuals dominate the remaining forest patches, CWD and FWD decreased further. CWD dropped to very low levels because trees hardly produced stems with diameters > 10 cm. We are not aware of other studies that quantitatively measured deadwood in tropical subalpine forests up to 4000 m.

In the alpine zone, CWD was completely absent and FWD also reached very low levels. Only few woody shrubs, such as *E. trimera agg* or *Euryops dacrydioides* contributed to the total deadwood pool and vegetation in general is rather scarce at this elevation.

Impacts of Land Use and Disturbance History on Deadwood

In the savanna zone, deadwood pools did not differ between natural plots and maize fields. This result was unexpected, since live tree biomass was significantly higher in natural plots than in maize fields (Ensslin et al., 2015). A very likely explanation is that people have gathered deadwood in our natural plots, probably for several decades. Human populations can reach high densities in this zone and local people rely heavily on wood as the only source of fuel (Mollel et al., 2017).

Even though people enter natural forest plots in the lower montane zone to collect deadwood, these plots harbored significantly more deadwood than homegardens, coffee plantations, and grasslands. The mean amount of total deadwood in this forest type lies within the range found in other tropical moist forests [14.7–25.4 Mg C ha^{-1}; Iwashita et al. (2013)].

Home gardens of local Chagga people contain some trees for shade, timber, and fruit provision (Hemp, 2006c), but all deadwood is immediately used as firewood. Therefore, both, CWD and FWD pools were small in homegardens. Commercial coffee plantations are managed monocultures with a tree cover of 10–30%. Few small twigs were the only source of deadwood. Most likely these plots are immediately cleaned up whenever deadwood appears, which would explain the low measured deadwood pool in this zone. Grasslands did not harbor any deadwood at all. This land-use type is maintained by frequent cutting of the herb layer for the provision of fodder (Mollel et al., 2017). Therefore, trees and shrubs are mainly absent.

In the montane zone, the CWD biomass of previously logged forests was twice as high as that of undisturbed forests. However, this difference was not statistically significant. The opposite pattern was observed for FWD; which occurred at more than double the rate in undisturbed forests than in logged forests (this was not statistically significant either). Strong increases in deadwood pools after logging were reported in many other studies (Gerwing, 2002; Keller et al., 2004; Feldpausch et al., 2005; Pfeifer et al., 2015). However, the time lags between the actual logging and the deadwood inventory in these studies did not exceed 9 years. In the montane zone of Mt. Kilimanjaro, however, logging has been prohibited since 1984 (Agrawala et al., 2003). Hence, this time span could have been long enough for regrowth to even out the differences in deadwood pools between formerly logged and unlogged forests. This is also supported by Ensslin et al. (2015), who found that live aboveground biomass did not differ significantly between logged and non-logged plots. Furthermore, Gourlet-Fleury et al. (2013) found that tropical forests in the Central African Republic fully recovered 24 years after logging. The reason for this higher amount of CWD in disturbed forests could be the growth habit of the dominant tree *Ocotea usambarensis*. After the cutting of large individuals, the stumps start to resprout resulting in several stems originating from one stump. Only a few of these stems survive, in particular the most erect ones, whereas most others with partly distinct inclination die after some decades. This resprouting is also reflected by a higher stem density in logged versus non- logged plots (Rutten et al., 2015a).

In the upper-montane zone, we did not find significant differences in CWD or FWD pools between undisturbed and formerly burned plots. This is at a first glance somewhat surprising, since burning has strongly changed species composition and forest structure (Hemp and Beck, 2001; Hemp, 2006b; Rutten et al., 2015b) at this altitude. Undisturbed upper montane *Podocarpus* forests have an upper canopy, which is 2–3 times taller than that of formerly (50 years ago) burnt *E. excelsa* succession stages. However, such *E. excelsa* forests consist of three times the number of stems, with this equalling the woody biomass of undisturbed forests (Hemp, unpublished data).

In the subalpine zone, undisturbed plots harbored significantly more CWD and FWD than plots, which have experienced burning in the past. Burning probably removed most of the woody biomass, dead or alive, and fires occurring with a high frequency degraded former *Erica* forests to shrublands (Hemp, 2005a). These anthropogenic activities affecting deadwood pools result also in the alteration of C and nutrient cycling by affecting microbial activities and enzymes catalytic properties in the soils of Kilimanjaro (Ndossi et al., 2020).

CONCLUSION

In this study, we showed that deadwood is an important structural component of different forest types at Mt. Kilimanjaro. We found that among our study sites, deadwood can account for up to 37.2 % of the total aboveground woody biomass and therefore also plays a considerable role in C sequestration. For this reason, we suggest that it should be considered in future programs for the reduction of emissions due to deforestation and degradation (REDD+; Burgess et al., 2010).

C content in wood samples increased with altitude at Mt. Kilimanjaro, suggesting that differences in climatic conditions in combination with shifts in species composition along environmental gradients influence the carbon pool of deadwood in tropical forests. Deadwood accumulation strongly followed the hump shaped relationship between elevation and live wood biomass accumulation with a peak at mid-elevations driven by the local precipitation scheme.

The combined effects of climate and decomposer communities on decomposition rates could be another reason for the observed hump-shaped pattern in deadwood stock along the elevation gradient of Mt. Kilimanjaro. We also found that anthropogenic land-use intensification at low to mid-elevations strongly reduced deadwood pools. Past selective logging in the montane zone slightly promoted the accumulation of CWD even after more than 30 years of recovery time and frequent fires in the subalpine zone removed most deadwood and degraded former forests into shrublands with only little woody biomass.

Deadwood remains a poorly understood, yet important, component of tropical forests and quantitative data sets are still rare. With this study we showed how deadwood accumulation and composition can depend on natural and man-made environmental gradients at Mt. Kilimanjaro. Further research, especially in the tropics, is needed to reach a more comprehensive understanding of the underlying drivers and role of dead woody biomass.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AK: writing, fieldwork, and analysis. AE: analysis. AH: fieldwork. MK: organization and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.732092/full#supplementary-material

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Distinct Community-Wide Responses to Forecasted Climate Change in Afrotropical Forests

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More refined knowledge of how tropical forests respond to changes in the abiotic environment is necessary to mitigate climate change, maintain biodiversity, and preserve ecosystem services. To evaluate the unique response of diverse Afrotropical forest communities to disturbances in the abiotic environment, we employ country-wide tree species inventories, remotely sensed climate data, and future climate predictions collected from 104 1-ha plots in the central African country of Gabon. We predict a 3–8% decrease in Afrotropical forest species richness by the end of the century, in contrast to the 30–50% loss of plant diversity predicted to occur with equivalent warming in the Neotropics. This work reveals that forecasts of community species composition are not generalizable across regions, and more representative studies are needed in understudied diverse biomes. This study serves as an important counterpoint to work done in the Neotropics by providing contrasting predictions for Afrotropical forests with substantially different ecological, evolutionary, and anthropogenic histories.

Keywords: afrotropics, Gabon, joint attribute modeling, biodiversity, community

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INTRODUCTION

The anticipated pace of global warming is predicted to result in large declines of tropical biodiversity (Aubry-Kientz et al., 2019), leading to biotic attrition of the lowland tropics (Sala et al., 2000; van Vuuren et al., 2006; Colwell et al., 2008; Feeley et al., 2011; Hooper et al., 2012; Dexter et al., 2018, but see Feeley and Silman, 2010). Effects of climate change may be indirect (Carley et al., 2021; Clark et al., 2021; Qiu et al., 2021), and most acutely felt by long-lived organisms like trees that endure several degrees of warming within a single lifetime, without the benefit of adaptive nimbleness available to shorter lived species (Malhi et al., 2014; Clark et al., 2021). Tropical forests contain over 40,000 tree species (Slik et al., 2015), shelter over half of all animal species (Pimm and Raven, 2000), and store much of the planet's carbon (Sullivan et al., 2017; Beirne et al., 2019; Poulsen et al., 2020) while covering only 7% of the Earth's surface (Corlett and Primack, 2011). Despite their outsize value, tropical forests are notably understudied, and most *in situ* species inventory data come from a few intensively studied sites in the Neotropics (Schimel et al., 2015; but see Blundo et al., 2021). Neotropical studies demonstrate that community composition and function are degrading in response to climate change (Engelbrecht et al., 2007; Bongers et al., 2009; Poorter et al., 2017; Whitworth et al., 2021), with early successional species thriving in warmer soil temperatures at the expense of late-successional species that require cooler microhabitats (Colwell et al., 2008). These processes have contributed to predictions of 30–50% loss of plant diversity with a 5°C temperature increase for most South American tropical forests (Colwell et al., 2008; Feeley and Silman, 2010).

It is unclear whether the world's other tropical regions will respond similarly to climate change (Malhi and Wright, 2004; Maslin et al., 2005; Parmentier et al., 2007; Malhi et al., 2013; Mayaux et al., 2013; Enquist et al., 2017; Sullivan et al., 2017). Afrotropical forests are distinct in having comparatively few wet-affiliated species given their climate (Leal, 2009), and a high proportion of large trees that grow and recolonize rapidly (Fayolle et al., 2012; Gond et al., 2013). These differences in community-level traits are hypothesized to have arisen from Africa's unique climatic past (Haffer, 1969; Maley, 1996; Maslin et al., 2005; Oslisly et al., 2013; Willis et al., 2013). In direct contrast to the Neotropics (Colinvaux et al., 1996, 2000, 2001), abnormally cool and dry conditions during the last glacial maximum reduced Afrotropical forests to small fragmented patches (Cohen et al., 2007). This may have selected for species able to survive extreme aberrations in temperature and precipitation and then quickly disperse from refugia to recolonize the landscape cleared by receding glaciers (Leal, 2009), potentially making them more resilient to climate change than their Amazonian or Asian counterparts (Hansen and DeFries, 2004; Gardner et al., 2007). Indeed, a pair of studies comparing changes in canopy structure found few lingering effects of drought on Afrotropical forest canopy (Asefi-Najafabady and Saatchi, 2013), but did find lingering canopy effects of drought on southwest Amazonia (Saatchi et al., 2013).

Neotropical species to climate change are unlikely to be an adequate proxy for Afrotropical forests, yet no landscape-scale predictions of Afrotropical community responses to climate have been made. This work is urgently needed—the current climate of Equatorial Africa is already near the lower temperature-precipitation threshold of rainforest viability (Malhi and Wright, 2004; Pan et al., 2011), after which a rapid shift in the ecosystem could occur (Willis et al., 2013). Accurate models of forest change in the Afrotropics are dependent on sampling both historical refugia and areas of expansion. Past reviews of paleoecological studies (Maley, 1996; Bonnefille, 2007) have interpreted the high degree of endemism in western Gabon relative to surrounding areas as an indicator that this area was an climatically stable refuge for tropical plants during the last glacial maxima. However, whereas recent genetic studies of some species support this claim (Dainou et al., 2010), still others indicate that these areas were not refuges for all species (Lowe et al., 2010, see also temperate examples: Comes and Kadereit, 1998; Petit et al., 2003), highlighting a need for systematically random census data in a topographically and abiotically diverse Afrotropical region.

To evaluate how Afrotropical tree species will respond to future climate change, we model tropical forest tree species distributions using a systematic country-wide array of randomly placed tree plots, remotely sensed historic climatic data, and future climate predictions for the densely forested central African country of Gabon. This first-of-its-kind study serves as an important counterpoint to work done in the Neotropics by providing contrasting novel predictions for Afrotropical forests with substantially different ecological, evolutionary, and anthropogenic histories. We hypothesize that the disturbance-rich past of Afrotropical communities will result in two divergent responses to forecasted climate change: (1) Afrotropical community forecasts will demonstrate lower levels of species loss than the 30–50% loss predicted for the Neotropics; and (2) early-successional species will increase in number at the expense of declining abundance of late-successional species.

MATERIALS AND METHODS

Tree Inventory Data

In this study, we employ tree census data from Gabon's National Resource Inventory—a national network of tree plots for estimating forest biomass and carbon stocks (**Figure 1**; Carlson et al., 2017; Poulsen et al., 2017a, 2020; Beirne et al., 2019; Wade et al., 2019). Gabon is the second most forested country in the world, with a forest cover of 87%, a deforestation rate near zero (Sannier et al., 2014), and one of the highest densities of carbon in Central Africa (Saatchi et al., 2011). Between 2012 and 2013, trained technicians established 104 1-ha forest plots based on a stratified random sampling design that consisted of dividing the country into 100 50 × 50 km cells and randomly locating a sample site within each of the cells (Poulsen et al., 2020). This design ensured an unbiased sampling of Gabon's forest (40.4% old-growth, 28.8% logged, 30.8% secondary) and edaphic types (69.2% terra firma, 22.1% seasonally flooded forest, 8.6% swamp). Every tree with a diameter-at-breast height (DBH) ≥ 10 cm was mapped, measured and identified to species by trained

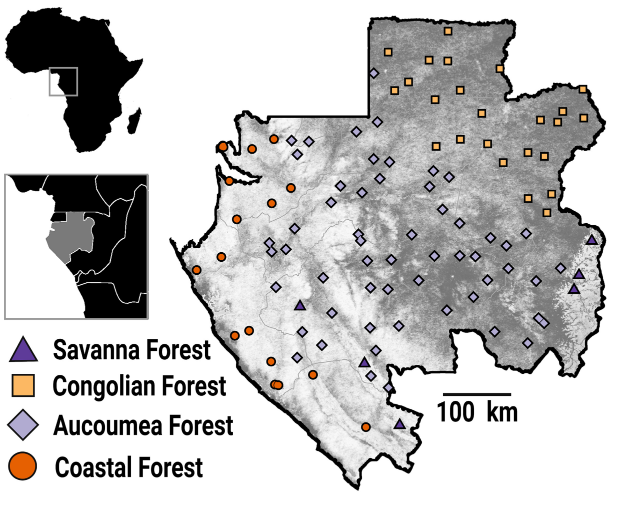

FIGURE 1 | Map of all 104, 1-ha inventory plots located in Gabon. Plots were located by a systematic-random design to capture the full breadth of Gabon's forest types and environmental conditions. Shapes indicate one of four possible forest-types of each plot: Savanna Forest (triangles), Congolian Forest (squares), Aucoumea Forest (diamonds), or Coastal Forest (circles).

field teams following standard protocols for plot establishment and measurement (Phillips and Baker, 2002). A total of 621 species from 296 genera were cataloged. We analyzed data for all species that occurred on at least 30 of the 104 plots, resulting in 34,460 stems representing 76 of the most widely occurring species. The five most common taxa (31% of all stems) were *Santiria trimera, Dichostemma glaucescens, Plagiostyles Africana, Aucoumea klaineana*, and *Diospyros* spp. The five least common taxa included in the model (1% of all stems) were *Duboscia macrocarpa, Ongokea gore, Zanthoxyllum heitzii, Klainedoxa* sp., and *Erythrophleum ivorense*. Each species was assigned, if possible, into an "early-successional" or "late-successional" class based on growth form, available trait data, and habitat class (Whitmore, 1989; Raich and Khoon, 1990; Finegan, 1996; Davies and Semui, 2006; Chazdon et al., 2010; Chazdon, 2014).

Climate Data

Long term average historical precipitation and temperature data for each plot were derived from the NASA TerraClimate product (Abatzoglou et al., 2018) for 1985–2017, accessed using Google Earth Engine (Gorelick et al., 2017). We derived projected precipitation and temperature data for each plot using the NASA Earth Exchange Global Daily Downscaled Projections (NEX-GDDP) database at a resolution of 0.25 degrees (∼25 km × 25 km). This dataset provides downscaled projections for two of the most used Representative Concentration Pathways (RCP 4.5 and RCP 8.5) from the 21 General Circulation Models that were produced and distributed under the Coupled Model Intercomparison Project Phase 5 (CMIP5). All models of temperature agree that Gabon will continue to warm through the end of the century; thus, within each RCP scenario, we took the ensemble mean prediction of all 21 CMIP5 GCMs to forecast of forest response (**Figures 2A,B**). Models of precipitation disagree as to whether precipitation will increase or decrease, producing an ensemble mean that shows no substantive change by 2099 (**Figure 2C**). This is due in large part to a lack of rain gauges in Central Africa (Washington et al., 2013), and also from the northward shift of the inter-tropical convergence zone resulting from ocean-driven atmospheric circulation shifts (James et al., 2013). To acknowledge the uncertainty in whether the dry or wet models are more plausible, here we forecast community change given both a wet and a dry scenario. The wet scenario takes the mean prediction of the five models predicting the greatest increase in precipitation, while the dry scenario uses the mean prediction from the five models predicting the greatest decreases in precipitation (**Supplementary Table 3**). Although representing the extreme scenarios, this forecasted climate space is well represented by historical climate space (**Supplementary Figure 1**).

Community Composition Analysis

We use a generative Generalized Joint Attribute Model (GJAM) that predicts species abundance at the scale and context used to fit the model jointly, i.e., on the community scale (Clark et al., 2017). GJAM estimates can therefore be interpreted on the scale of the observations, accounting for sample effort. Full model specifications are available from Clark et al. (2017). Parameters

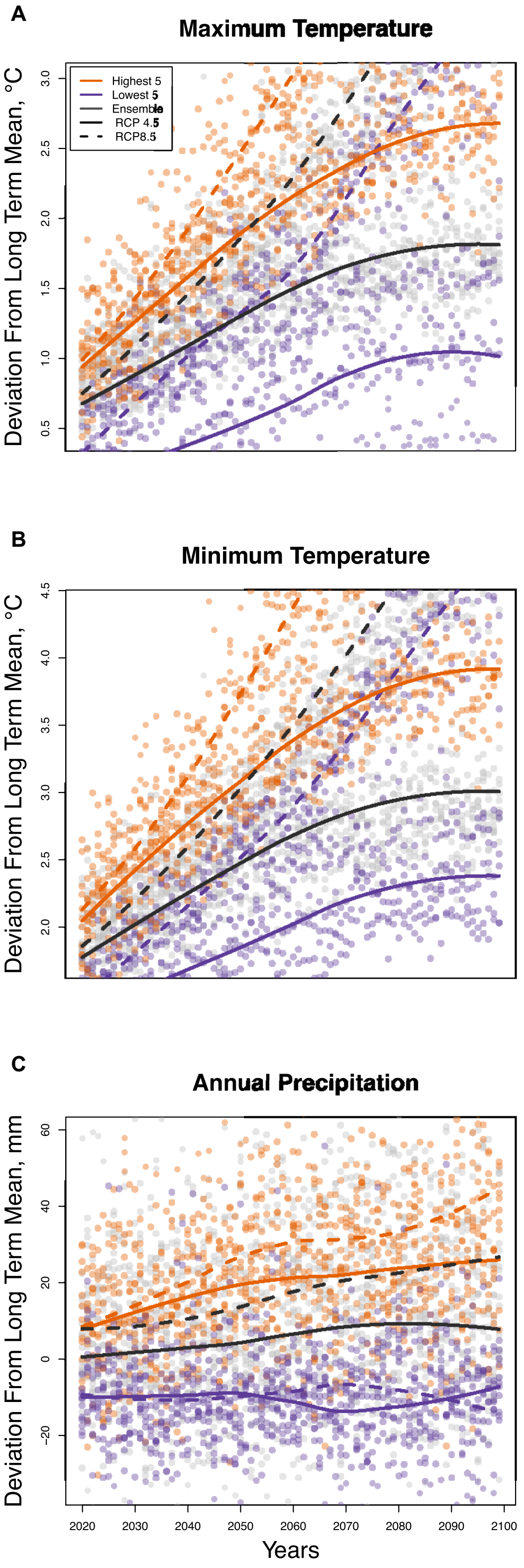

FIGURE 2 | A comparison of average predicted maximum temperature **(A)**, minimum temperature **(B)**, and precipitation **(C)** in Gabon from the highest temperature increase models (orange), lowest five models (purple), and all model ensembles (gray) for RCP 4.5 (solid lines) and RCP 8.5 (dashed lines).

in the model include matrices of coefficients B relating X to Y and the residual species covariance matrix Σ. In effect, Σ represents the covariance between species beyond what is explained by environmental covariates. This variation can come from interactions between species, unmeasured environmental variables, and other sources of error. The likelihood is: $[Y_1,\ldots,Y_S|X, B, \Sigma]$, where subscripts refer to species 1 through S. Model fitting is done on the observation scale, and is based on the posterior distribution, $[B, \Sigma|X,Y] \propto [Y1,\ldots,YS|B, \Sigma][B, \Sigma]$. The right-hand side of the equation is the likelihood and the prior distribution, $[B, \Sigma]$, which is non-informative. Traditional species distributions models cannot generate the covariance structure in Y or the proper uncertainty for B because they are not fitted jointly, i.e., because $[Y_1, \ldots, Y_S\,|B, \Sigma\,, X]$ is not equal to likelihood of independent SDMs, $[Y_1\,|B, \Sigma\,, X] \times \ldots \times [Y_S\,|B, \Sigma\,, X]$. SDMs predict each species independently, which ignores their interactions. Joint modeling is frustrated by the different scales of measurement for many species and dominance of zeros in most data sets. GJAM allows for continuous and discrete data, including zeros, observed on different scales and with different levels of sample effort.

Forecasting Species Composition

The predictive distributions combine the posterior parameter estimates calibrated from long-term climate data with a prediction grid of forecasted covariate values (precipitation and temperature). X^* is a vector of environmental covariates that generate a response vector of species $Y^* = Y_1, \ldots, Y_S$, and (X^*, Y^*) is a pair of vector observations used to fit the model. The predictive distribution $[Y^*\,|X^*] = \int [Y^*\,|B, \Sigma, X^*]\,[B, \Sigma\,|X, Y]\,d\,(B, \Sigma)$ is obtained by Monte Carlo integration. The two factors in the integrand are the likelihood and posterior distribution. The two factors in the integrand are the likelihood and posterior distribution. If the prediction is based on an uncertain scenario for climate X^*, that uncertainty enters the prediction as $[Y^*] = \int [Y^*\,|X^*]\,[X^*]\,dX^*$. In this analysis, $[X^*]$ is a prior distribution that is uniform over the full range of values that have been observed.

To quantify how changes in climate will affect total species counts regardless of forest type, we first run the model using forest type (Congolian, savanna, coastal, Acoumea) as a random effect. We then use forest type as a factor to make predictions about species counts within forest types. Covariates used to fit the model were limited to those for which there are predictions available from the NASA NEX GDDP GCM's for years 2020–2099 and having variance inflation factors less than 3. Model estimates were taken from 100,000 iterations, discarding the first 1000 iteration as pre-convergence. We visually inspected trace plots to confirm convergence and adequate mixing (**Supplementary Figure 3**) and validated model fit by comparing predicted and observed discrete species abundances (**Supplementary Figure 2**).

RESULTS

Of the 76 most abundant species analyzed, species richness in Gabon's forests is projected to decrease by 3–8% by the end of the century, although there is variation among precipitation models and RCP scenarios (**Figures 3**, **4** and **Supplementary Table 1**). The dry model predicts a loss of 2.58 focal species per plot ± SE 5.36 in the low emissions scenario (RCP 4.5, **Figure 4A**), and −5.85 ± 5.12 species in the high emissions scenario (RCP 8.5, **Figure 4C**). The wet model predicts fewer species losses (RCP 4.5: −3.17 ± 5.37; RCP 8.5: −5.96 ± 5.11, **Figures 3A,C**). Species losses were consistent across forest types

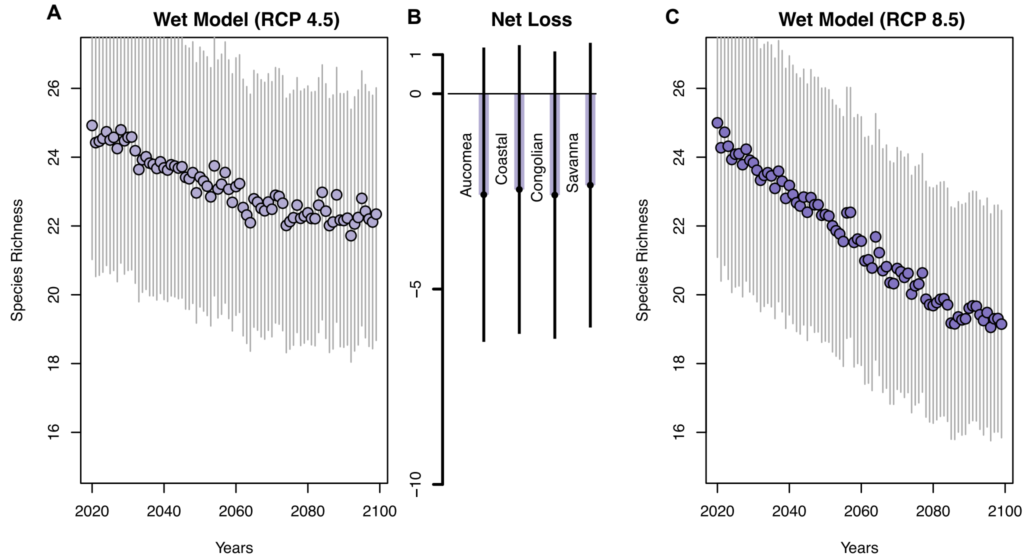

FIGURE 3 | Declines in estimated plot-level species richness and the estimated net tree species loss for each forest type by the end of the century for RCP 4.5 **(A,B)** and RCP 8.5 **(C,D)** for the wet model.

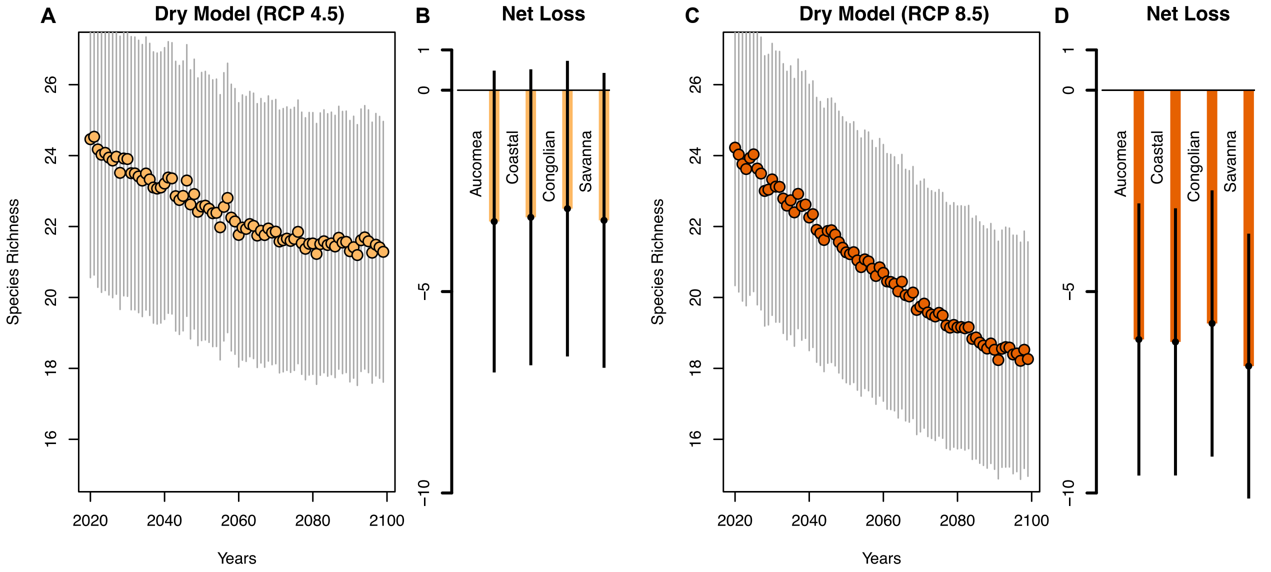

FIGURE 4 | Declines in estimated plot-level species richness and the estimated net tree species loss for each for each forest type by the end of the century for RCP 4.5 **(A,B)** and RCP 8.5 **(C,D)** for the Dry model.

(**Figures 3B,D**, **4B,D** and **Supplementary Table 2**) with greatest losses in the RCP 8.5 scenario (**Figures 3D**, **4D**) compared to RCP 4.5 (**Figures 3B**, **4B**). Of the 76 species we analyzed approximately one third are predicted to increase in abundance and two-thirds of species likely to decrease in abundance. The most likely species to increase in abundance include *Diospyros* spp., *Aucoumea klaineana*, and *Staudia gabonensis*. By contrast, *Santira trimera*, *Plagiostyles africana,* and *Dichostemma glaucescens* are predicted to decrease in abundance (**Figure 5**). Net change in predicted species abundance was similar between wet and dry models and among RCP scenarios (**Figure 5** and **Supplementary Table 4**).

DISCUSSION

We demonstrate a 3–8% decrease in Afrotropical forest species richness by the end of the century for the most abundant tree species. This prediction is substantially less severe than 30–50% predicted for the Neotropics (Colwell et al., 2008; Feeley and Silman, 2010), and lends support to the argument that the unique evolutionary past of Afrotropical forest communities (Malhi and Wright, 2004; Maslin et al., 2005; Parmentier et al., 2007; Malhi et al., 2013; Mayaux et al., 2013; Enquist et al., 2017; Sullivan et al., 2017) could have made them more resilient to climate change than their Amazonian or Asian counterparts (Hansen and DeFries, 2004; Gardner et al., 2007). It also indicates that predictions of tree species responses to climate change are not generalizable across continents. Surprisingly, we find that losses do not differ appreciably among forest types, indicating that no habitats will be immune from species changes. The equivalent responses may also be a result of the subjective distinction between our four forest types—communities are complex assemblages of species that may not always readily lend themselves to clear classification.

We find that species-specific changes in abundance varied considerably, with approximately a third of all species likely increasing in abundance and two-thirds of species likely decreasing in abundance. Our hypothesis that disturbance-related species would increase at the expense of climax species was not well supported. Of the ten species most likely to *increase in abundance*, only two are considered early successional. Of the ten species most likely to *decrease in abundance*, five were early successional. It is interesting that the species increasing are not just those that are fast growing, low wood density species associated with disturbance. For example, two of the species most likely to increase are *Acoumea klaineana* (a light-loving, low wood density species), and *Diospyros* spp., the genus that incudes ebony (high wood density, slow growing). Among the species expected to decrease in abundance is *Santira trimera*, one of the most widespread species throughout West and Central African rainforests, often in moist secondary forests or along rivers. Also predicted to decrease is *Dichostemma glaucescens,* a small slender tree prone to climbing other trees. These species-specific responses suggest that rather than functional groups responding similarly, tropical species respond individualistically to tropical climate change. This pattern agrees with past research (Bush, 2002; Bush et al., 2004) and may arise from unique relationships to unmeasured abiotic variables that contribute to its response to disturbance (Nuñez et al., 2019a). A comparison of the forecasts produced by both dry and wet models yields surprising little difference in predicted species richness. Although the total water available to trees are the product of both precipitation and temperature, these results suggest that species will respond more strongly to increases in temperature, not precipitation as it is in the

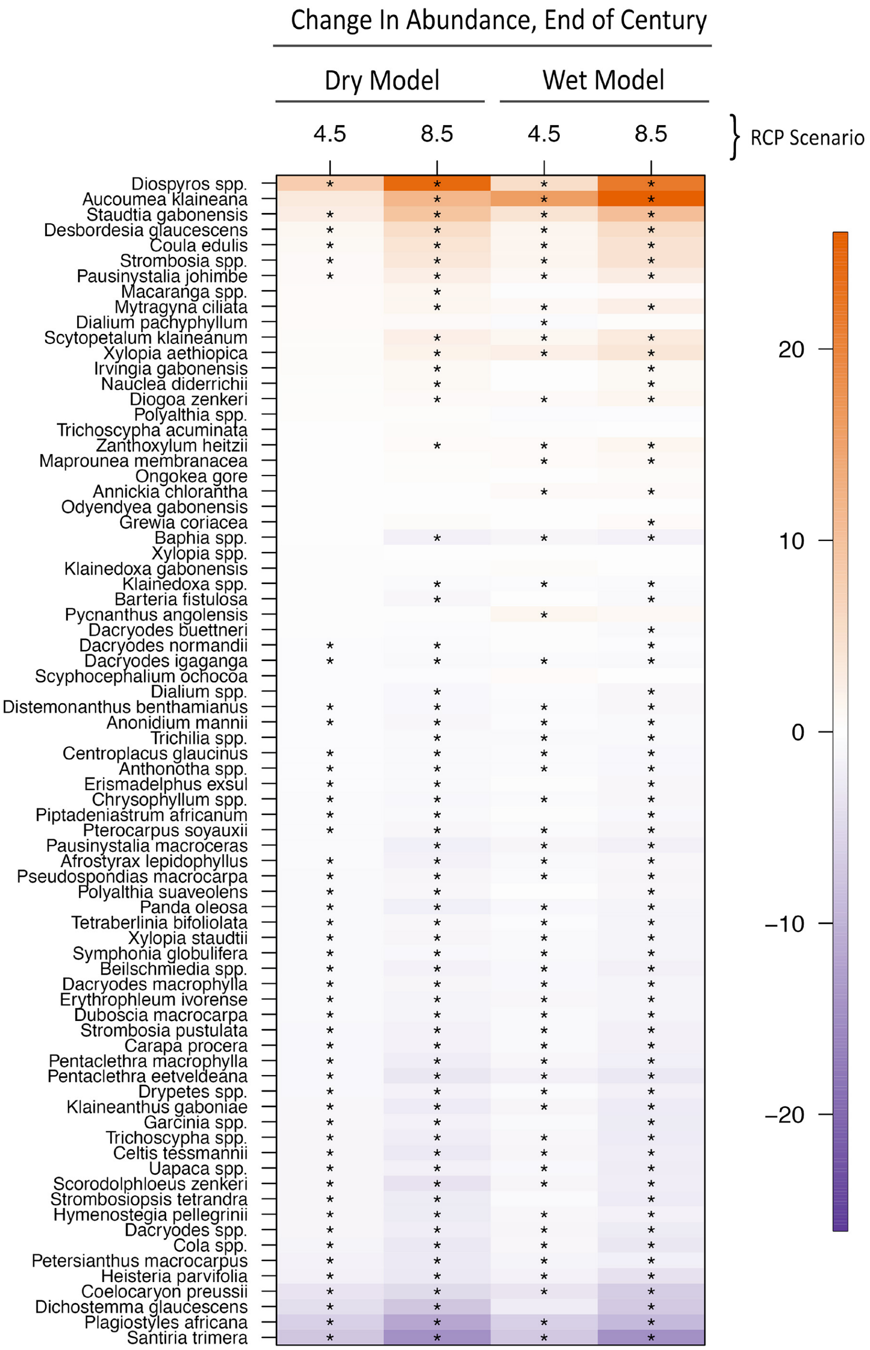

FIGURE 5 | Predicted change in abundance for each tree species (y-axis) by the end of the century for RCP 4.5 and RCP 8.5 in both the "wet" and "dry" model ensembles (x-axis). Box colors correspond to estimated changes in species abundance. "*" symbol in box denotes species-model predictions that do not overlap with 0.

Neotropics. For this reason, the increasing radiative forcing associated with RCP 8.5 resulted in greater species loss than the more conservative RCP 4.5 scenario. Temperature specific responses are consistent with the theory that a unique ecological history in the Afrotropics (Malhi and Wright, 2004; Maslin et al., 2005; Parmentier et al., 2007; Malhi et al., 2013; Mayaux et al., 2013; Enquist et al., 2017; Sullivan et al., 2017) cultivated tree communities with few wet-affiliated species (Leal, 2009).

Several considerations are necessary to situate this study in the literature. First and foremost, this analysis considered only the 76 most common species across plots. Rare species (occurring on fewer than 30/104 plots) have few observations and provide insufficient information on their relationship with environmental predictors to make accurate predictions, perpetuating the "rare species modeling paradox" (Lomba et al., 2010). This could mean that we are ultimately underestimating losses of total species richness because it is precisely these rare species that are most vulnerable to climate change (Ohlemüller et al., 2008; Pacifici et al., 2015). However, limiting analyses to species with adequate data is a common component of many analyses, including those predicting 30–50% loss in the Neotropics (Wisz et al., 2008; Feeley and Silman, 2010).

The model predicts at the scale of the data, i.e., the community level, allowing a comparison of a species likelihood of presence in a plot with full uncertainty. However, the model does not explicitly consider mechanistic changes in recruitment, carbon enrichment, seedling survival, or changes in dispersal. As such, the model does not account for plasticity that may allow for species to occur in plots outside their current climate space. Although such mechanistic understanding is needed, the type and resolution of data currently available make this impossible. These results do not consider other types of disturbance that are likely to affect biodiversity, but are less clearly measurable, like the loss of medium and large animals from hunting (Koerner et al., 2017; Poulsen et al., 2017b, 2018, 2021; Beirne et al., 2019; Nuñez et al., 2019c). Finally, although the geographic scope of this analysis is uncommonly expansive, covering the entire country of Gabon (267,667 km^2), it may not be directly comparable to the diverse terrain contained in the 2,250 km^2 assessed in the Neotropics (Feeley and Silman, 2010; Maicher et al., 2021). Care must also be taken when generalizing our results—although the impacts predicted here appear to be modest relative to the new world Tropics, elsewhere in sub-Saharan Africa has already seen devastating effects of climate change (Willis and Bhagwat, 2009; Willis et al., 2013; Serdeczny et al., 2017).

This study demonstrates that community forecasts are not generalizable across regions, and more studies are needed in understudied biomes like the Afrotropics. Nascent data sets (Enquist et al., 2017; Fyllas et al., 2017), increased availability of high quality remote sensing (Patterson and Healey, 2015; Stavros et al., 2017; Silva et al., 2018), and new statistical techniques capable of synthesizing multiple types of data (Clark et al., 2017) will help in further resolving the responses of the world's ecosystems. This study serves as an important counterpoint to work done in the Neotropics by providing contrasting predictions for Afrotropical forests with substantially different ecological, evolutionary, and anthropogenic histories. Even though we are predicting a comparatively small reduction in species richness, the effects reported here will have ramifications for whole food webs (Dirzo et al., 2014; Clark et al., 2019), and potentially threaten the ecosystem services on which humans depend (McCann, 2000; Hooper et al., 2005; Balvanera et al., 2006; Cardinale et al., 2012; Schweiger et al., 2018). The differences exposed by this work should serve as motivation for future research using fine scale data to compare the differing responses of tropical biomes to global change.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data are subject to third party restrictions. The data that support the findings of this study are available from Le Ministère des Eaux, de la Forest, de la Mer, de l'Environnement. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the corresponding author with the permission of Le Ministère des Eaux, de la Forest, de la Mer, de l'Environnement. Requests to access these datasets should be directed to JP, john.poulsen@duke.edu.

AUTHOR CONTRIBUTIONS

CN conducted the analysis and wrote the manuscript. CN, JP, VM, and LW designed the experiment and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.742626/full#supplementary-material

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Biogeographical Importance of the Livingstone Mountains in Southern Tanzania: Comparative Genetic Structure of Small Non-volant Mammals

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The Livingstone Mountains (LM; also known as the Kipengere Range) found in south-western Tanzania at the northern end of Lake Nyasa are an important region for understanding the biogeography of Eastern Africa. The two branches of the East African Rift Valley meet here and the mountains might represent stepping stones for colonization and migration between different parts of the Eastern Afromontane Biodiversity Hotspot (especially the link between the Eastern Arc Mountains, EAM, and the Southern Rift Mountains, SRM), as well as an efficient barrier to gene flow for taxa living in drier savannahs in lower elevations. Here we combine new mitochondrial sequence data from 610 recently sampled rodents and shrews with available georeferenced genetic data (3538 specimens) from southern Tanzania, northern Malawi/Zambia and northern Mozambique and compare the spatial genetic structure among different taxa. There is no universal phylogeographic pattern in taxa preferring humid montane habitats. For some of them, the Makambako Gap acts as a barrier between the SRM and the EAM, but other taxa can bridge this gap. Barriers within the EAM (frequently) and within the SRM (sometimes) appear more important. The Rukwa rift between the SRM and the ARM is an important barrier that perhaps can only be crossed by taxa that are not that strictly tied to humid montane environments. For mammals living in lower-elevation savannah-like habitats, the LM can act as a strict barrier to gene flow, and together with the Ufipa Plateau, Lake Nyasa and the EAM create a very similar phylogeographic pattern with three recognizable genetic groups in most savannah-dwellers. The Livingstone Mountains thus appear to be one of the most important biogeographic crossroads in Eastern Africa.

Keywords: comparative phylogeography, rodents, shrews, Eastern Arc Mountains, Southern Rift Mountains, Eastern Afromontane Biodiversity Hotspot, savannah

INTRODUCTION

The Eastern Afromontane Biodiversity Hotspot (EABH) is the biodiversity hotspot with the second highest number of endemic higher vertebrate genera, after Madagascar and the Indian Ocean islands (Mittermeier et al., 2011). It is estimated to be home to at least 7,598 plant species (2,356 endemic), 1,325 bird species (157 endemic), 490 mammal species (104 endemic), 347 reptile species (93 endemic) and 323 amphibian species (100 endemic) (Mittermeier et al., 2004; Birdlife International, 2012). These numbers are underestimates as new surveys continue to discover new species (Rovero et al., 2014) and genotyping efforts continue to uncover cryptic diversity (Demos et al., 2014; Greenbaum et al., 2015; Pozzi et al., 2020). These mountains are often thought of as islands of humid forest surrounded by drier lowland savannahs and woodlands (White, 1983). However, the montane forest islands were not always as isolated from each other. It is generally accepted that in the early Miocene forests likely spanned from the west to the east coast of Africa (Coetzee, 1993). Later on, the climate became drier in eastern Africa due to substantial tectonic uplift. The forests started to fragment and the EABH forests were separated from the Guineo-Congolian forests (Sepulchre et al., 2006). Subsequent climatic oscillations in the Plio-Pleistocene were crucial for forming contemporary species/genetic diversity in the EABH. Dry and humid climatic phases alternated, contracting and expanding forests (Williamson, 1985; Trauth et al., 2007). During humid cycles, forest expansion may have connected humid forests of different mountains ranges or has at least facilitated dispersal between them, e.g., through the riverine forests (see Couvreur et al., 2021 for more details).

The EABH corresponds to the elevated regions of the East African Rift: from the Asir Mountains of southwest Saudi Arabia and the highlands of Yemen in the north; along the Eastern Rift branch including the Ethiopian Highlands, the Kenyan and Tanzanian Highlands and the Eastern Arc Mountains (EAM); and along the Western Rift branch including the Albertine Rift Mountains (ARM), the Southern Rift Mountains (SRM) and distant outliers in the Chimanimani Highlands of eastern Zimbabwe and Gorongosa of western Mozambique (Mittermeier et al., 2004; **Figure 1A**). The Eastern and Western Rift branches meet at the northern end of the Southern Rift Mountains, the so-called Livingstone Mountains (LM; also known as the Kipengere Range; **Figure 1B**). The LM are comprised of the Mbeya Range north of Mbeya, the Uporoto Mts. (including Mt. Ngozi) in the west, Mt. Rungwe, the Kitulo Plateau (formerly known as the Elton Plateau), the Kipengere Range (in the stricter sense) in the east and the Livingstone Mountains (in the stricter sense), including the Livingstone Escarpment along the north-eastern coast of Lake Nyasa, in the southwest (**Figure 2**).

Small non-volant mammals (rodents and shrews) are very suitable models for reconstructions of historical biogeography, because they are relatively abundant and easy to capture, their dispersal capacity is limited and their affinity to particular types of habitats is very high. The unique location of the LM at the crossroads of the SRM and the intensively studied and species-rich ARM and EAM makes this understudied region a crucial area to improve our understanding of the biogeographic history of eastern Africa. Recently we performed multiple field expeditions and collected small mammals both in and around the LM. The focus of this paper is to compare phylogeographic patterns of small mammals living both in moist highland ecosystems and in drier savannahs and woodlands that surround them. Comparison of multiple mammalian taxa rather than focusing on one or two allows a more general picture on past habitat connectivity to be formed, as particular taxa might show different patterns due to variability in their habitat specificity, diet, dispersal ability etc. Specifically, we assess: (1) how unique the LM small mammals are in comparison with those on other montane islands of the SRM, the EAM and the ARM; and (2) the role of these mountains in forming a barrier to gene flow in savannah-dwelling taxa. The aim of this study is not to perform any taxonomic analysis or to test the role of any mechanisms behind the evolution of current biodiversity, but to compare general patterns in the distribution of genetic variability that have likely been created by changes in connectivity of montane forests and/or savannahs due to past climatic changes from the Pliocene onward.

MATERIALS AND METHODS

Sampling

In order to assess the role of the LM in connecting different parts of the EABH and as a barrier to gene flow for savannah/woodland taxa, we used numerous georeferenced and genotyped specimens, 3538 published and 610 newly sampled or sequenced (**Supplementary Material 1**). The distribution of genetic diversity was analyzed for 17 taxa (at the level of closely related species and/or intraspecific lineages), which represent a significant part of assemblages of rodents and shrews that can be trapped by the use of standard live- and snap-traps (only few rarely sampled taxa were omitted because of the lack of material). In 2013 we collected small mammals in a montane forest and its margins on Mt. Ngozi using snap traps and Sherman live traps (H.B. Sherman Traps Inc., Tallahassee, United States). In 2018 and 2019 we captured small mammals in the Livingstone Escarpment and in the neighboring Kyela Plain (lowland at the north-western tip of Lake Nyasa at the foot of the Livingstone scarp) by Sherman live traps baited with a mixture of peanut butter and maize flour. Traps were usually set in and on the edge of agricultural fields in lines of 10 traps with equal distance in between or in half-grids of 5 by 10 traps. Live-caught small mammals were euthanized by cervical dislocation or an overdose of Isoflurane prior to dissection. Samples are stored in 96% ethanol at the Institute of Vertebrate Biology (IVB) of the Czech Academy of Sciences and at the University of Antwerp (UA). The field work was approved by the UA Ethical Committee for Animal Experimentation (2014-98 and 2017-75) and complied with regulations of the Research

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Abbreviations: AM, African Mammalia (database); ARM, Albertine Rift Mountains; *CYTB, cytochrome b;* EABH, Eastern Afromontane Biodiversity Hotspot; EAM, Eastern Arc Mountains; LM, Livingstone Mountains; SRM, Southern Rift Mountains.

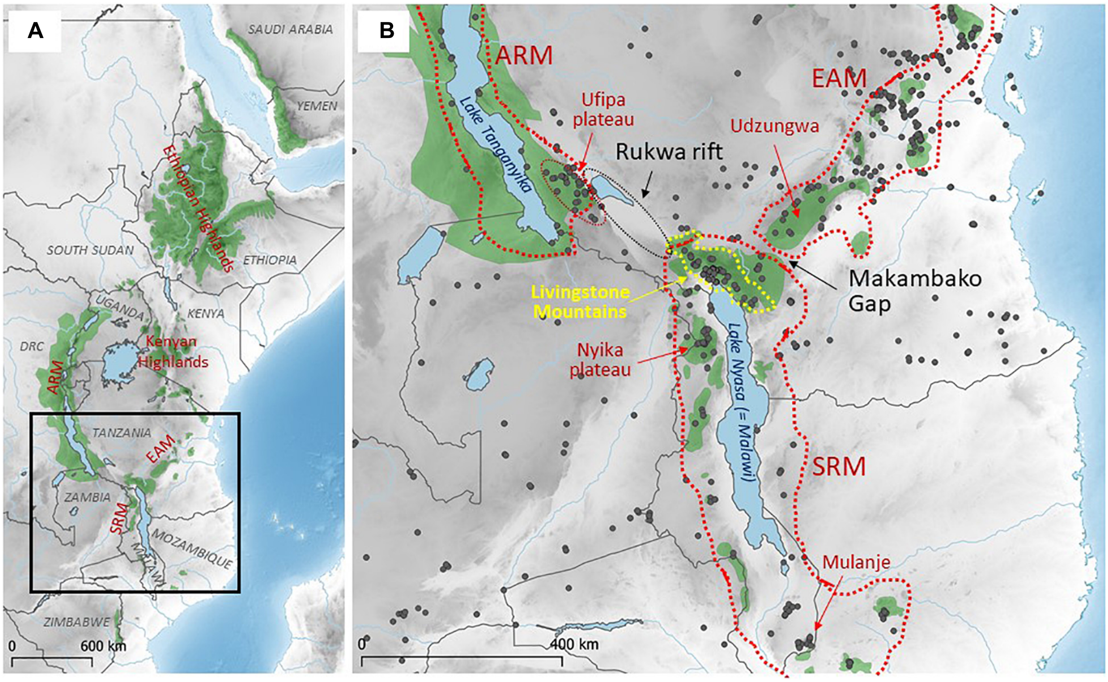

FIGURE 1 | (A) Map of the Eastern Afromontane Biodiversity Hotspot (in green; following Birdlife International, 2012). Its most important larger blocks are the Ethiopian Highlands, Albertine Rift Mountains (ARM), Kenyan Highlands, Eastern Arc Mountains (EAM) and Southern Rift Mountains (SRM). **(B)** Detailed map of the study area, i.e., the Livingstone Mountains (LM) (yellow dotted line) and neighboring highland ecosystems (in green) and lowland savannah-like habitats. Gray dots are positions of genotyped small mammals relevant to this study. For a comparative analysis of highland taxa, we included samples from the SRM, the EAM and the southernmost part of the ARM. Two important gaps in the current distribution of montane ecosystems are marked by black arrows (i.e., areas of lowland savannahs, called the Rukwa rift and the Makambako Gap). For a comparative analysis of savannah/woodland taxa, we used data collected from these habitats in Tanzania, Zambia, Malawi and Mozambique.

Policy of Sokoine University of Agriculture as stipulated in the Code of Conduct for Research Ethics. Because of geographical proximity, we also summarized unpublished data available in the African Mammalia database (Van de Perre et al., 2019) on animals collected on Mt. Rungwe in 1987 by W. Verheyen, J. Stuyck, and M. Colyn (**Figure 2**).

Genotyping and Phylogenetic Analysis

The analysis of genetic diversity is primarily based on the comparison of mitochondrial (mt) DNA sequences. There is a growing database of rodent and shrew mtDNA sequences for the *cytochrome b* gene (*CYTB*), enabling comparative analyses of genetic structure. Even though *CYTB* is only one non-recombining locus, which might introgress into other evolutionary lineages (e.g., between rodent taxa in geomorphologically complex Ethiopian Highlands; Bryja et al., 2018b; Komarova et al., 2021), it is an informative marker to explore genetic structure in the studied area, as demonstrated by comparison of mtDNA structure with that obtained by nuclear markers (Bryja et al., 2014b, 2017; Krásová et al., 2019; Onditi et al., 2021) or even genomic approaches (our unpublished data). Therefore, we performed comparative analysis of phylogeographic structure using *CYTB* as a main marker. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) for the 610 newly sampled small mammals and the complete *CYTB* gene (1,140 bp) was amplified using L14723 and H15915 primers (Lecompte et al., 2002) according to the protocols described in Bryja et al. (2014b). The sequences were aligned with all relevant published data (including those in GenBank and the African Mammalia database; Van de Perre et al., 2019). The alignments are available upon request, new sequences were submitted to GenBank (accession numbers OK093418-OK094020). For a list of all included georeferenced and genotyped samples (see **Supplementary Material 1**).

Species and intraspecific clades in particular groups of small mammals were identified by ML analysis in RAxML 8.2.8 (Stamatakis, 2014), using the GTRCAT substitution model as suggested by the authors of the program. The robustness of the nodes was evaluated by the default bootstrap procedure with 1,000 replicates and new sequences were considered as part of previously defined taxa/clades if the bootstrap support was > 95%. Specifically, we compared the new sequences with relevant data in recent phylogenetic studies (Castiglia et al., 2012; Colangelo et al., 2013; Bryja et al., 2014a,b; Stanley et al., 2015; Bryja et al., 2017, 2018a; Mazoch et al., 2018; Petružela et al., 2018; Sabuni et al., 2018; Krásová et al., 2019, 2021; Hánová et al., 2021a,b; Onditi et al., 2021; Dianat et al., unpublished data) and/or with unpublished sequences obtained by our teams in East Africa in the last decade. The distribution maps of genetic

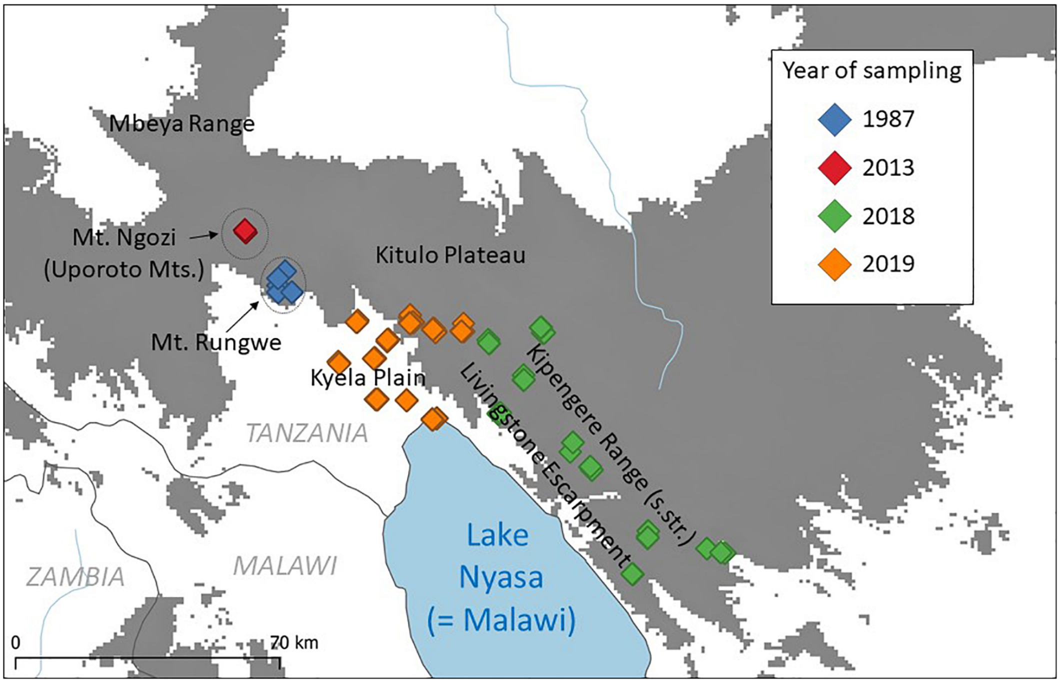

FIGURE 2 | New localities sampled within this study in and around the Livingstone Mountains. Most samples were collected on Mt. Ngozi (in the Uporoto Mountains) in 2013 (red), and in the Livingstone Escarpment and the Kyela Plain at its base in 2018 (green) and 2019 (orange). We also present unpublished data from Mt. Rungwe (blue; collected in 1987 by W. Verheyen, J. Stuyck and M. Colyn) that is available online on the African Mammalia database (Van de Perre et al., 2019). Elevation above 1,500 m a.s.l. is colored in gray.

clades were created using the Free and Open Source QGIS v. 3.16 (QGIS.org, 2021).

RESULTS

Small Mammals of the Livingstone Mountains

The field collections during four expeditions in the LM comprised 992 small mammals, classified into 4 shrew and 20 rodent species (**Table 1**). They represent taxa living in a wide spectrum of habitats. Besides the taxa typical for montane forests (*Montemys*, *Hylomyscus*), forest margins (*Mus triton*, *Lophuromys*, *Grammomys*) and humid grasslands (*Rhabdomys*, *Dendromus*, *Crocidura montis* group), we also collected taxa living in savannahs (*Mus minutoides*, *Saccostomus*, *Acomys*, *Crocidura hirta/flavescens*). The combination of newly collected genetic data with recently published phylogeographical studies thus allows assessment of the role of the LM in creating patterns of genetic diversity in both highland and lowland types of ecosystems (summarized in **Figures 3**, **4**). We did not include taxa with very limited data or taxa without georeferenced and genotyped samples in the LM area (with the exception of *Heliophobius* and two species of *Aethomys* that were not captured during our survey, but are very likely to live in the area). Two species, *Lemniscomys striatus* and *Crocidura hildegardeae*, are reported for the first time in this area and these records push the boundary of their known distribution range more southwards.

Comparative Genetic Structure of Taxa Living in Humid Highland Habitats of the Livingstone Mountains

The distribution of evolutionary clades of taxa living in relatively humid highland habitats (montane forest and their margins) is summarized in **Figure 3**. There is no universal pattern across the eight analyzed taxa. The *Montemys delectorum* clade occurring in the LM is also found in SRM in northern Malawi. A very distinct clade occurs in the southern outliers of the SRM and two more clades are found in the EAM, one from the Udzungwa Mts. up to the Uluguru and Ukaguru Mts. and a second in the Usambara Mts. and northern Tanzanian highlands (**Figure 3A**). Another montane forest specialist, *Hylomyscus arcimontensis*, probably has a more restricted distribution (i.e., it is not recorded in the northern Tanzanian highlands and the southernmost part of the SRM) and the break from one clade to another occurs within the LM rather than between the LM and EAM. The Ufipa plateau (Mbizi Mts., already part of the ARM) is inhabited by another species from the same species group, *H. stanleyi* (**Figure 3B**).

The following species are not strictly associated with forests, but they live in the mosaic of highland forests and open moist grassy habitats. The SRM *Rhabdomys dilectus dilectus* are divided in three clades, one in the LM, which also occurs in the

TABLE 1 | Small mammals collected in the Livingstone mountains and the Kyela plain (see **Figure 2** for sampled localities).

Species	Mt. Rungwe	Mt. Ngozi	Livingstone Escarpment + Kyela Plain	
	1987[a]	2013	2018	2019
N total	298	47	211	436
SHREWS				
Soricidae				
Crocidura hildegardeae	–	–	–	2
Crocidura hirta	–	–	–	8
Crocidura luna	–	5	1	–
Crocidura turba	–	–	–	1
RODENTS				
Bathyergidae				
Fukomys whytei[b]	10	–	–	–
Nesomyidae				
Dendromus nyassae[c]	9	–	3	–
Saccostomus campestris	–	–	–	2
Muridae				
Acomys muzei	–	–	–	2
Dasymys incomtus[d]	–	–	2	2
Gerbilliscus boehmi	–	–	1	–
Grammomys surdaster[e]	2	–	8	3
Hylomyscus arcimontensis[f]	3	–	–	4
Lemniscomys striatus	–	–	–	5
Lophuromys machangui	85	17	9	44
Mastomys natalensis	23	–	75	266
Montemys delectorum[g]	72	21	3	15
Mus minutoides[h]	7	–	11	–
Mus triton	69	4	78	64
Otomys lacustris	1	–	–	–
Pelomys falax	1	–	–	–
Rattus rattus	3	–	–	–
Rhabdomys dilectus	10	–	20	18
Gliridae				
Graphiurus sp.	1	–	–	–
Sciuridae				
Paraxerus lucifer	2	–	–	–

[a] *No data about shrews are present in the African Mammalia (AM) database from the Mt. Rungwe expedition, but it is possible that they were collected.*
[b] *As Cryptomys hottentotus in AM (but see Faulkes et al., 2017).*
[c] *As D. insignis in AM (but see Voelker et al., 2021).*
[d] *The taxon from the LM and neighboring highlands was separated into a separate species D. alleni by Verheyen et al. (2003), but we continue to use the older name D. incomtus awaiting a more detailed phylogenetic study.*
[e] *As Grammomys dolichurus in AM (but see Bryja et al., 2017).*
[f] *As Hylomyscus stella in AM (but see Kerbis Peterhans et al., 2020).*
[g] *As Praomys delectorum in previous studies (but see Nicolas et al., 2021).*
[h] *As Mus musculoides in AM (but see Bryja et al., 2014b).*

Udzungwa Mts. (EAM), one in the Nyika Plateau and one in the Mulanje Mts. (**Figure 3C**). The *Lophuromys flavopunctatus* group (sometimes also called *L. aquilus* group) has been split into several species with a parapatric distribution in the EABH. *Lophuromys machangui* lives in the SRM (including the LM) and the Udzungwa Mts, while *L. kilonzoi* lives in the rest of the EAM, *L. sabuni* in the southern part of the ARM including the Ufipa Plateau, and *L. laticeps* more north in the ARM (**Figure 3D**). Three clades of *Dendromus nyasae*, living in highland grasslands, have a very similar distribution to three species of *Lophuromys* (**Figure 3E**). *Crocidura montis* clade 3 (i.e., *C. luna*) was captured in the SRM, including the west of the LM. In the east of the LM, the ARM and in the Udzungwa Mts., *C. montis* clade 1 was captured. A further three species from the *Crocidura montis/monax* group from the EAM were delimited by integrative taxonomic analysis, *C. munissi*, *C. tansaniana,* and *C. usambarae* (**Figure 3F**).

The last two species are more opportunistic, and besides montane forests they can also occupy drier habitats, such as miombo woodland. Most *Mus triton* caught in the LM belong to the same clade as those found in the EAM, but two individuals caught in 1987 around Mt. Rungwe belong to the same clade as those found in the other SRM and the ARM (**Figure 3G**). An individual captured east of the LM forms a distinct genetic clade. For *Grammomys surdaster*, we clustered the mtDNA clades described in Bryja et al. (2017) into four groups based on unpublished ddRAD analyses (Bryja et al., unpublished data). The widespread genomic multilocus clade found in the ARM and Zambia occurs also in the SRM, including the LM, and Udzungwa. A separate group was found in lower elevations of the western LM and east of the LM and Udzungwa. Two additional genetic clades inhabit the southernmost part of the SRM and the central and northern EAM (**Figure 3H**).

Comparative Genetic Structure of Taxa Living in Savannahs and Woodlands Around the Livingstone Mountains

For savannah and woodland taxa around the LM, the patterns are more uniform. In most taxa, there are three genetic groups, even though their genetic distances are not comparable across taxa (as suggested in the original phylogenetic-taxonomic publications, for more details see **Supplementary Material 2**): (1) a group in north-western/central Tanzania; (2) a group in south-eastern Tanzania and north-eastern Mozambique; and (3) a group in Zambia, Malawi, the rest of Mozambique and part of south-western Tanzania (**Figure 4**). The groups (1) vs. (2) often appear separated by the EAM, (1) vs. (3) by the LM and/or the Ufipa Plateau, and (2) vs. (3) by Lake Nyasa and the LM. For example, in the *Crocidura hirta/flavescens* complex, *C.* cf. *flavescens* occurs north of the EAM, the LM and on the Ufipa Plateau, *C. hirta* clade I occurs around the EAM and east of the LM and Lake Nyasa, and *C. hirta* clade II occurs southwest of the LM and south of the Ufipa Plateau (**Figure 4E**). Another clear example is *Heliophobius argenteocinereus* where the N clade is found north of the EAM, northwest of the LM and in the ARM including the Ufipa Plateau, the SE clade is found south east of the EAM, the LM and Lake Tanganyika; and the SW clade is found southwest of the LM, west of Lake Nyasa and more to the south (**Figure 4B**).

There are some variations on this general pattern. For example, in several taxa, the distribution of the south-western (*M. natalensis*, *A. chrysophilus*, *S. campestris*, *A. kaiseri*) or south-eastern (*L. rosalia*) group extends up until the Ufipa Plateau (**Figures 4A,F–I**, respectively), where it can meet the northern clade (in *M. natalensis*, **Figure 4A**). For some taxa,

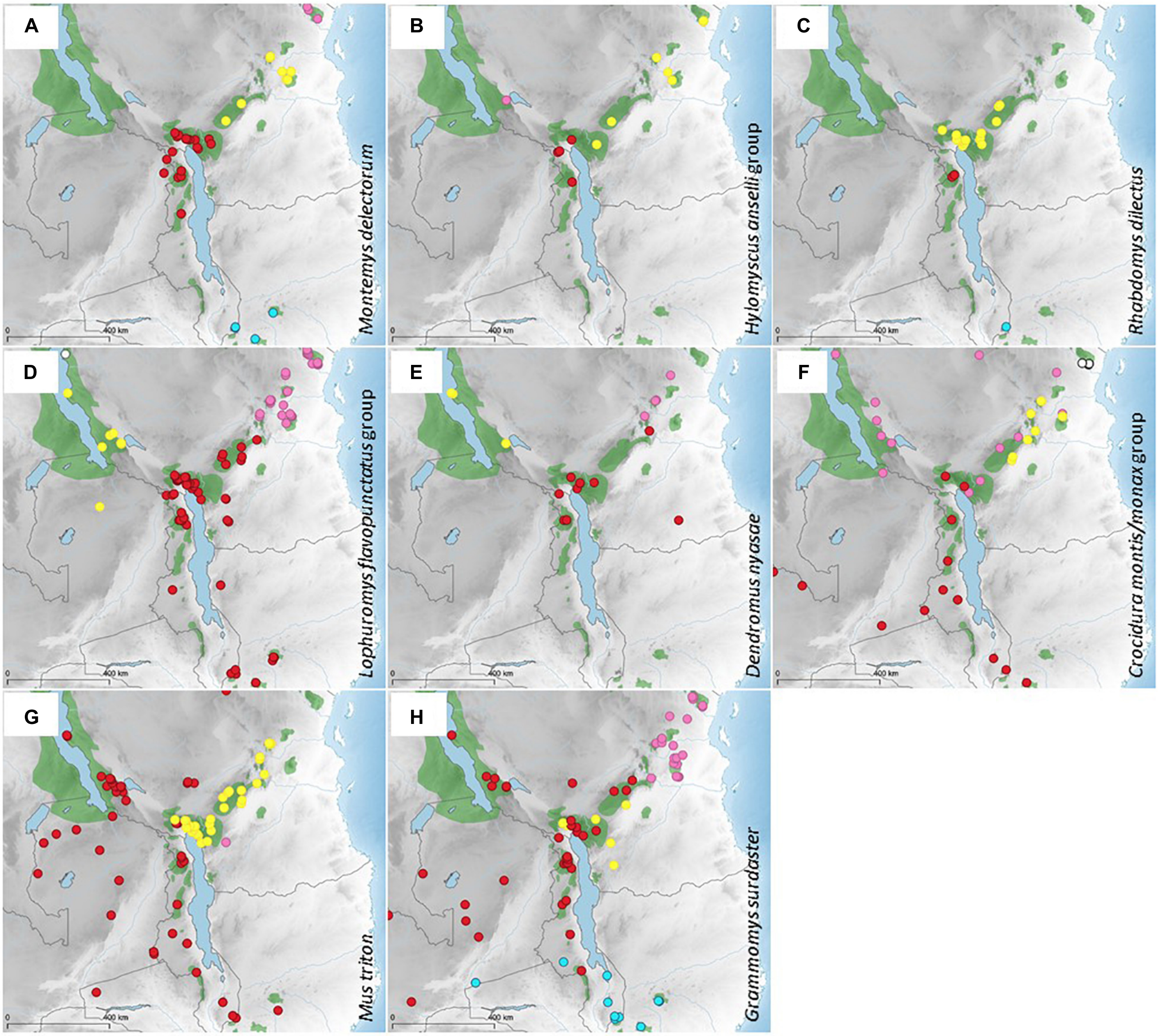

FIGURE 3 | Distribution of genetic clades of eight taxa of small mammals living in humid montane forests and their margins in the Livingstone Mountains. Published data were complemented by new records from the studied area and reanalyzed. For more details (data source, GPS coordinates, GenBank accession numbers, etc.) see **Supplementary Material 1**. Different symbols/colors represent genetic clades delimited as follows: **(A)** *Montemys delectorum* (based on Bryja et al., 2014a and Sabuni et al., 2018: blue—*M. delectorum* clade A, red—*M. delectorum* clade B1, yellow—*M. delectorum* clade B2, pink—*M. delectorum* clade C). **(B)** *Hylomyscus anselli* group (based on Kerbis Peterhans et al., 2020; Nicolas et al., 2020: pink—*H. stanleyi*, yellow—*H. arcimontensis* clade 1, red—*H. arcimontensis* clade 2). **(C)** *Rhabdomys dilectus dilectus* (based on Castiglia et al., 2012; Sabuni et al., 2018: yellow—*R. d. dilectus* clade A1, blue—*R. d. dilectus* clade A2, red—*R. d. dilectus* clade A3). **(D)** *Lophuromys flavopunctatus* group (based on Sabuni et al., 2018; Onditi et al., 2021: red—*L. machangui*, pink—*L. kilonzoi*, yellow—*L. sabunii*, white—*L. laticeps*). **(E)** *Dendromus nyasae* (based on Voelker et al., 2021: yellow—*D. nyasae* clade 1, red—*D. nyasae* clade 2, pink—*D. nyasae* clade 3). **(F)** *Crocidura montis/monax* group (based on Stanley et al., 2015; Sabuni et al., 2018: pink—*C. montis* clade 1, red—*C. montis* clade 3, i.e., *C. luna*, yellow—*C. munissii*, white—*C. tansaniana*, blue—*C. usambarae*). **(G)** *Mus triton* (based on Krásová et al., 2019: yellow—*M. triton* clade C, red—*M. triton* clade D, pink—*M. triton* clade E). **(H)** *Grammomys surdaster* (based on Bryja et al., 2017 and modified on the basis of unpublished genomic ddRAD analysis: red—*G. surdaster* clades su3, su4, su7, su8-North grouped together based on ddRAD, yellow—*G. surdaster* clade su9, blue—*G. surdaster* clade su8-South, pink—G. *surdaster* clade su10, except for the southernmost locality that has mtDNA su9, but clearly groups with the pink clade at nuclear genomic data).

only two groups can be distinguished with current data. For example, the same clade of *Aethomys chrysophilus* is found in southern Tanzania, Zambia, Malawi and Mozambique, as opposed to northern and central Tanzania (**Figure 4F**). For the *Acomys spinosissimus* group, *A. muzei* is found in north-western Tanzania, Zambia and Malawi, as opposed to *A. ngurui* in south-eastern Tanzania and Mozambique (**Figure 4C**). However, if we split the internal Tanzanian crown clade m4 of *A. muzei* from the

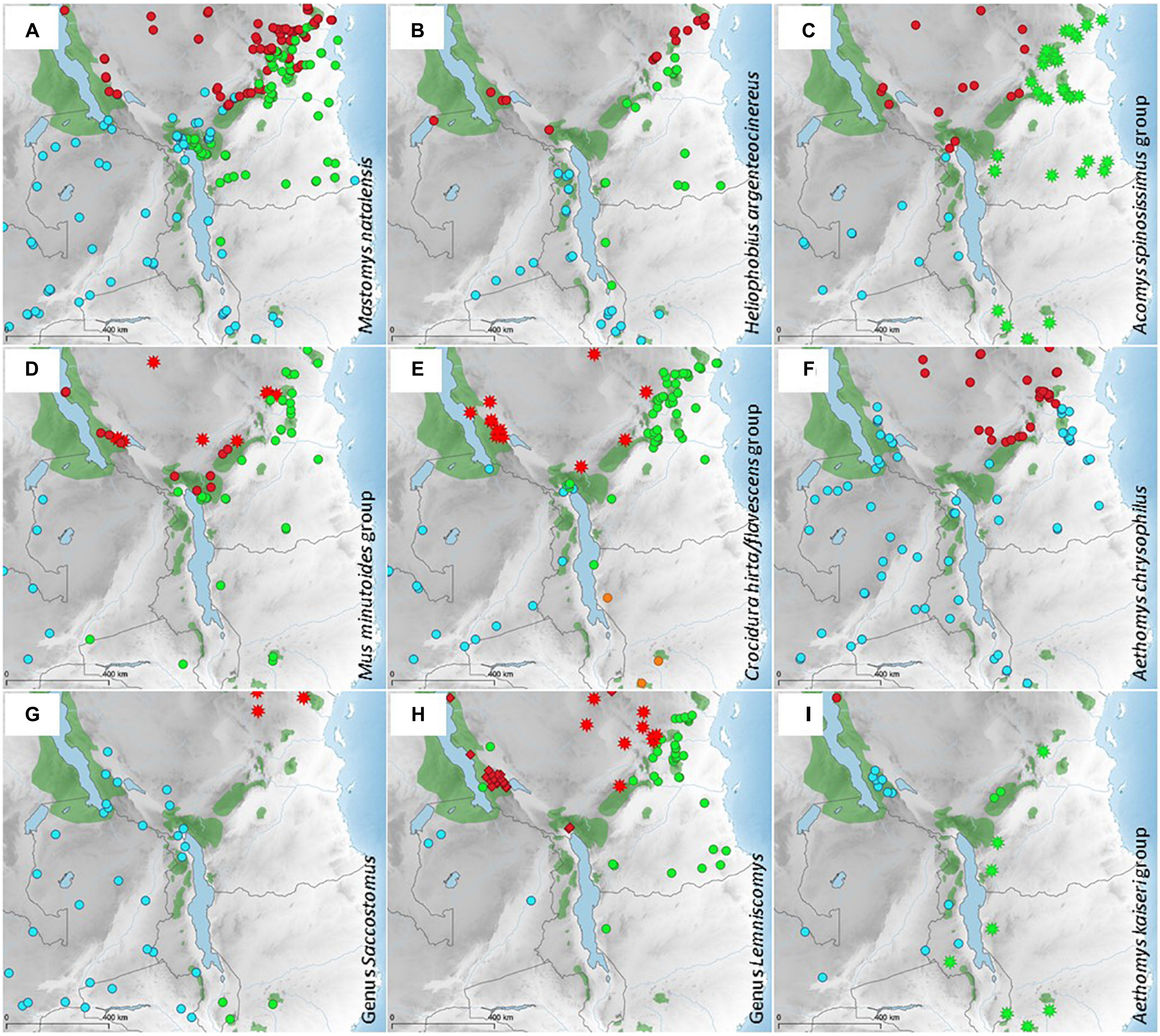

FIGURE 4 | Distribution of genetic clades of nine taxa of small mammals living in savannahs and woodlands (e.g., miombo or mopane woodlands) around the Livingstone Mountains (LM). Published data were complemented by new records from the studied area and reanalyzed. For more details (data source, GPS coordinates, GenBank accession numbers, etc.) (see **Supplementary Material 1**). Different symbols/colors represent the following genetic clades: **(A)** *Mastomys natalensis* (based on Colangelo et al., 2013; Hánová et al., 2021a): red—*M. natalensis* clade B-IV, green—*M. natalensis* clade B-V, blue—*M. natalensis* clade B-VI). **(B)** *Heliophobius argenteocinereus* (from Bryja et al., 2018a; Uhrová et al., 2021: red—*H. argenteocinereus* clade N, green—*H. argenteocinereus* clade SE, blue—*H. argenteocinereus* clade SW). **(C)** *Acomys spinosissimus* group (based on Petruželа et al., 2018: red—*A. muzei* clade m4, blue—*A. muzei* other clades, green stars—*A. ngurui*). **(D)** *Mus minutoides* group (based on Bryja et al., 2014b: red—*M. minutoides* clade TZw, green—*M. minutoides* clade SE, blue—*M. minutoides* clade ZA, red stars—*M.* cf. *gerbillus*). **(E)** *Crocidura hirta/flavescens* complex (based on Dianat et al., unpublished data: red—*C.* cf. *flavescens*, green—*C. hirta* clade I, blue—*C. hirta* clade II, orange—C. hirta clade III). **(F)** *Aethomys chrysophilus* (based on Mazoch et al., 2018: red—*A. chrysophilus* clade E, blue—*A. chrysophilus* clade G). **(G)** *Saccostomus* (based on Mikula et al., 2016: blue—*S. campestris campestris* clade B, green *S. campestris campestris* clade C, red stars—*S. umbriventer*. **(H)** *Lemniscomys* (based on Hánová et al., 2021b: blue—*L.* cf. *roseveari*, green—*L. rosalia* clade A, red rhombuses—*L. striatus* clade F, red stars—*L. zebra* clade B). **(I)** *Aethomys kaiseri* group (based on Krásová et al., 2021: blue—*A. kaiseri* clade I, green—*A. kaiseri* clade II, red—*A. kaiseri* clade III, green stars—*A. silindensis*.

other *A. muzei* clades, we do see the typical geographical pattern again. Two generalist species were also caught in agricultural fields in the LM. For *M. natalensis* a different clade was captured on the Livingstone Escarpment compared to the area around Mt. Rungwe and on the Kyela Plain. For *M. minutoides* one clade is observed rather central on the Livingstone Escarpment, while another occurs more south and at a locality on the Kyela Plain (**Figure 4D**).

DISCUSSION

The Livingstone Mountains: Diversity Hotspots Within the Eastern Afromontane Biodiversity Hotspot

Compared to the extremely rich biodiversity of ARM and EAM, the SRM are generally considered less diverse (Mittermeier et al., 2004). However, the LM as the northernmost tip of the SRM are uniquely positioned at the crossroads of all three mountain blocks, which may substantially increase diversity of its biota. While the humid Afromontane forests of the EABH are currently isolated in an archipelago-like fashion, faunal exchanges likely took place during more humid periods of the Pleistocene, allowing range expansion and gene flow in taxa adapted to moist habitats currently restricted to patches at higher elevations (e.g., Bryja et al., 2014a). The LM could therefore have acted as stepping stones for the colonization/migration between different parts of the EABH and they still harbor a mosaic of (intra) specific clades from the neighboring EABH complexes (**Figure 3**). There is no simple pattern of today's genetic structure across different taxa. As small mammals vary in many different traits, such as level of habitat specialization and dispersal ability, they have reacted differently to the same climate-driven habitat shifts and certain geographic features might have been a barrier to some, but not to others. It should be noted that several small mammals living in the mosaic of humid highland habitats (i.e., those shown in **Figure 3**) were also caught in the edges of agricultural fields. The genetic structure of more opportunistic species that also might live outside of montane forests such as *M. triton* or *G. surdaster*, already seems less spatially segregated compared to taxa with more specific habitat requirements such as *M. delectorum*, *H. arcimontensis* or the *L. flavopunctatus* group.

Despite differences among individual taxa, the comparison of genetic structure of multiple species living in the same sky islands in the EABH allows identification of geomorphological features that have been important as barriers to gene flow in the past. Here we assume a barrier if a given taxon only occurs in the LM, but not in the neighboring mountain ranges. Conversely, a taxon that occurs in the LM and in the ARM and/or EAM shows connectivity between those mountain ranges in the past.

The Makambako Gap is an unforested, dry, low-lying region covered by miombo woodland separating the LM (i.e., the SRM) and the EAM (**Figure 1B**). It has been shown to be an important barrier for a range of taxa, such as Tornier's cat snake (Gravlund, 2002), some bush vipers (Menegon et al., 2014), double-bearded chameleons (Ceccarelli et al., 2014), mountain greenbuls (Roy et al., 1998, reviewed in Kahindo et al., 2007; Fjeldså and Bowie, 2008), olive woodpeckers (Fuchs et al., 2021), and Tanzanian vlei rats (Taylor et al., 2009), but not for others, e.g., Uzungwe Mountain Bush Vipers (Menegon et al., 2014), forest batis (Fjeldså et al., 2006, reviewed in Kahindo et al., 2007; Fjeldså and Bowie, 2008), green barbets (Fjeldså and Bowie, 2008; Fjeldså et al., 2010) and Kihaule's mouse shrews (Stanley and Esselstyn, 2010). Similar conclusions can be drawn from our review of small mammals. The Makambako Gap has probably been (and currently clearly is) a Quaternary barrier to *M. delectorum* and some, but not all clades of the *C. montis/monax* group. On the other hand, *H. arcimontensis*, *R. dilectus dilectus*, *L. machangui,* and *M. triton* clade C do bridge the gap. Furthermore, some of our clades (*R. dilectus dilectus* clade A1, *M. triton* clade C and *G. surdaster* clade su9) only occur in the LM and the Udzungwa Mts., but were not trapped further south. This is also the case for certain bush vipers (Menegon et al., 2014), kipunjis (Davenport et al., 2006), and *Kinyongia msuyae* chameleons (Menegon et al., 2015). It should also be noted that the savannahs separating the central and northern EAM are often more important in forming the genetic structure of highland taxa than the Makambako Gap (**Figure 3**; see Fjeldså and Bowie, 2008 for a review of bird taxa).

The Rukwa rift is a grassland and woodland basin, separating montane habitats of the ARM from the SRM (**Figure 1B**). For small terrestrial mammals, the fauna of southern part of the ARM is very distinct from eastern mountains (i.e., the EAM and the SRM). They share the same mitochondrial lineages only in relatively opportunistic taxa that can live also in higher-elevation miombo woodlands, e.g., *M. triton*, *G. surdaster* and the *C. montis/monax* group. The situation is different in more specialized taxa living in high-elevation humid habitats. The genera *Rhabdomys* and *Montemys* do not occur in the ARM at all (the latter is replaced there by its ecological equivalent *Praomys jacksoni;* Mizerovská et al., 2019). The SRM species in the genera *Hylomyscus* and *Lophuromys* are replaced in the ARM by phylogenetically distant congeneric species (Kerbis Peterhans et al., 2020; Onditi et al., 2021). In other taxa, the phylogenetic affinities probably also depend on the level of specialization to montane forests. The Rukwa rift appears to have been a barrier for some mountain greenbuls (Roy et al., 1998; reviewed in Kahindo et al., 2007), the *Cinnyris afer* complex (Bowie et al., 2016), some bush vipers (Menegon et al., 2014) and the epiphytic plant *Canarina eminii* (Mairal et al., 2017), but not to Tanzanian vlei rats (Taylor et al., 2009) and Mt. Rungwe bush vipers (Menegon et al., 2014).

The Livingstone Mountains: Drivers of Diversification in Low Elevation Savannahs and Woodlands

While an expansion of moist montane forests during more humid periods of the Pleistocene likely connected taxa living in these forests and their edges, at the same time it effectively isolated the taxa living in the surrounding savannahs and woodlands (Colangelo et al., 2013; Mazoch et al., 2018; Petružela et al., 2018). These allopatric taxa could then diverge from each other in their respective "savannah refugia" and meet again during drier periods, such as the present. This seems to have occurred for a wide range of taxa living in open savannah-like habitats, e.g., ungulates (Lorenzen et al., 2012), baboons (Dunn et al., 2013), and fiscal shrikes (Fuchs et al., 2011). Moreover, the geographical areas where the clades that diverged in allopatry come into secondary contact, are roughly the same for many taxa and may therefore represent suture zones. In eastern Africa these zones are mostly located perpendicular to the main mountain ranges in the north-south direction (**Figure 4**). However, despite geographical coincidence across multiple taxa, there is variability in the level (i.e., time) of divergence, and the taxa have not diverged to the same extent. As a result, their secondary contact ranges from free admixture to limited hybridization to reproductive isolation (Lorenzen et al., 2012).

In our data we indeed observe that the humid Afromontane mountains are/were a barrier to gene flow for taxa living in drier, savannah-like ecosystems. Even though some clades are currently in contact, the EAM, the LM, Lake Nyasa and the Ufipa Plateau appear to have separated many taxa into three genetic groups (**Figure 4**). The different clades often represent intraspecific variation (indicated by dot symbols in **Figure 4**) driven by relatively recent Pleistocene climate oscillations, but in some cases, they represent well-distinguished species (indicated by stars or rhombuses). In two groups, the *Acomys spinosissimus* group and the *Aethomys kaiseri* group, the most distinct taxa (*A. ngurui* and *A. silindensis*, respectively) are limited to south-eastern savannahs/miombo woodlands (Petružela et al., 2018; Krásová et al., 2021; green stars in **Figures 4C,I**). However, this pattern is not the most prevalent. Environmentally speaking the ecological conditions are most distinct in the so-called Masai steppe in central Tanzania, and accordingly, the northern (red in **Figure 4**) groups are phylogenetically more distinct in many taxa. This is the case for *Mus* cf. *gerbillus* (vs. three intraspecific clades of *M. minutoides*; Bryja et al., 2014b; **Figure 4D**), *Crocidura* cf. *flavescens* (vs. three intraspecific clades of *C. hirta*; Dianat et al., unpublished data; **Figure 4E**), *Saccostomus umbriventer* (vs. two intraspecific clades of *S. campestris campestris*; Mikula et al., 2016; **Figure 4G**), *Lemniscomys striatus* and *L. zebra* (very distinct from two lineages of the *L. griselda* group, i.e., *L. cf. roseveari* and *L. rosalia*; Hánová et al., 2021b; **Figure 4H**). In two additional taxa (*Heliophobius* and *Aethomys chrysophilus*), the northern group is not yet taxonomically separated into a different species, but the northern populations are genetically and morphologically the most distinct and deserve a separate species status (Mazoch et al., 2018; Uhrová et al., 2021). The most striking exception from this pattern is *Acomys muzei*, which has a very high genetic diversity in Malawi and Zambia (Petružela et al., 2018). One internal clade of this species relatively recently colonized western and central Tanzania (**Figure 4C**). No other *Acomys* sp. are present there, so it may have filled an empty niche. Whether or not this colonization went hand in hand with adaptations to more arid environments is a hypothesis worth testing.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank, OK093418-OK094020.

ETHICS STATEMENT

The animal study was reviewed and approved by the UA Ethical Committee for Animal Experimentation (2014-98 and 2017-75).

AUTHOR CONTRIBUTIONS

LC, RŠ, SJEB, JGB, and JB conceived the study. HL, SJEB, JGB, and JB provided funding. LC, CS, RŠ, TA, EL, JGB, and JB collected the material. LC and TA performed genetic analyses. LC and JB analyzed the data and wrote the first draft of the manuscript that was complemented by all authors. All authors also approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.742851/full#supplementary-material

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Biodiversity and Ecosystem Functions Across an Afro-Tropical Forest Biodiversity Hotspot

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Ecosystem functions are important for the resilience of ecosystems and for human livelihood quality. Intact habitats and heterogeneous environments are known to provide a large variety of ecosystem functions. Natural and near natural ecosystems surrounding agroecosystems may positively support crop growing conditions and thus facilitate crop yields. In contrast, monocultures of crops and trees as well as degraded landscapes are known to provide less ecosystem functions. The Taita Hills in southern Kenya are part of the Eastern Afromontane biodiversity hotspot, and represent a habitat mosaic consisting of largely intact cloud forests, agroecosystems and plantations of exotic trees. In this region, subsistence farmers rely on ecosystem functions provided by natural ecosystems. In this study, we analyze three proxies of biodiversity and ecosystem functions, namely pollination activity, predation rates, and arthropod diversity in tree canopies. We set study plots along forest-agroecosystem-gradients, covering cloud forest, forest edge and agricultural fields, as well as plantations of exotic trees. We assessed environmental conditions, to evaluate the extent to which local environmental factors influence ecosystem functions. Based on these data we investigate potential spill over of ecosystem functions from forest into adjoining agroecosystems. For predation rates we found trends of spill over effects from forest interior into the agroecosystem. The expression of ecosystem functions differed among habitat types, with comparatively high predation rates in the forest, high pollinator activity in the open agricultural areas, and highest arthropod diversity along the forest edge. Eucalyptus plantations showed reduced ecosystem functions and lowest arthropod diversity. Local factors such as vegetation cover and flower supply positively influence pollinator activity. Our study show that natural ecosystems may positively contribute ecosystem functions such as predation, while the homogenization of biota through planting of invasive exotic tree species significantly reduce biodiversity and ecosystem functions. Transition habitats such as forest margins, and small-scale ecological enhancement positively influences biodiversity and ecosystem functions.

Keywords: pollination, predation, arthropod diversity, environmental conditions, ecosystem functions, habitat destruction, spill over, invasive exotic species

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INTRODUCTION

Natural ecosystems provide valuable habitats for numerous animal and plant species and ecosystem functions (Ebeling et al., 2018). Ecosystem functions are of high relevance to the resilience of ecosystems, and for human well-being (Naeem et al., 2010). Human livelihood quality rely on intact nature and landscapes (Millennium Ecosystem Assessment, 2005; IPBES, 2019). In the meanwhile, the value of ecosystem functions has been estimated and quantified in monetary terms in various studies (Dainese et al., 2019). For example, it is estimated that the annual pollination of crops by various insects alone creates US$ 195 billion to US$ 387 billion United States dollars in value (Klein et al., 2008; Porto et al., 2020). Studies evidenced that landscape homogenization in agroecosystems lead to a significant reduction of ecosystem functions and services, such as pollinator activity and pest control (Steffan-Dewenter et al., 2006; Witt et al., 2018; Dainese et al., 2019). Thus, natural and near-natural ecosystem interspersed throughout landscape may significantly increase crop yields in neighboring fields through positive spill over effects (Kollmann et al., 2016; Barrios et al., 2018; Dawson et al., 2019; Tamburini et al., 2020; Tschartke et al., 2021). This was evidenced for pollinators (Garibaldi et al., 2016) and predators like birds which are of high relevance for pest control (Benjamin et al., 2016; Karp et al., 2018; Tela et al., 2021).

The distribution of biodiversity and ecosystem functions become particularly evident for the highly degraded cloud forest fragments of Taita Hills, southern Kenya. This mountain range is part of the Eastern Afromontane biodiversity hotspot (Burgess et al., 2007; Mittermeier et al., 2011). These mountains were originally covered by cloud forest, but become almost completely deforested in the wake of human settlement (Maeda et al., 2010). Diverse and species riche cloud forest has been almost completely transformed into eucalyptus plantations and agricultural fields, interspersed by some few cloud forest remnants (Teucher et al., 2020), with negative effects on (endemic) flora and fauna (Omoro et al., 2010; Norfolk et al., 2017) as well as ecosystem functions (e.g., soil fertility, soil erosion, Michelsen et al., 1996; Scott et al., 2005; Baohanta et al., 2012; water availability, Hohenthal, 2018; Castro-Díez et al., 2019). For instance, many of the original local springs dried up during the past decades in the wake of large-scale planting of eucalyptus trees (Hohenthal et al., 2015). Other ecosystem functions such as pollination and pest control (among others, see Lamarque, 2009; Pellikka et al., 2013) might similarly negatively affected from ecosystem destruction.

In our work we measured different proxies of ecosystem functions in parallel, on identical plots and along gradients to test for potential spill over effects (from forest interior into the surrounding agroecosystems). Apart from measuring ecosystem functions on the ground, we also considered arthropod diversity of tree canopies, which harbor an essential part of biodiversity in tropical forests (see Nadkarni, 1994). And, in addition to potential effects and trends at a landscape scale (comparing different ecosystems), we also measured potential effects of the local environment for each study plot. For our analyses we considered natural cloud forest, forest edge (transition zone between cloud forest and agricultural land), agricultural fields (open land), and eucalyptus plantations. Ecosystem functions were measured at 10 × 10 m study plots, which were mainly set along gradients from forest interior, transgressing the forest edge, and ranging into the agricultural fields (perpendicular to the forest edge) (see Habel and Ulrich, 2020). We measured three proxies to quantify biodiversity and ecosystem functions: pollination activity, predation rates [according the Rapid Ecosystem Function Assessment (REFA) protocol, Meyer et al., 2015; Meyer et al., 2017], as well as arthropod diversity in tree canopies using flight interception traps (see Habel et al., 2021). We chose these proxies as they are considered to play a key-role for successful agriculture (Meyer et al., 2015) and represent the ecological performance of a landscape. In addition, we measured environmental parameters for study plots to account for potential local effects of environmental conditions on ecosystem functions. Based on these data and the results obtained, we will answer the following questions:

1. Are there significant differences in arthropod diversity and ecosystem functions between the different habitat types studied?
2. Do arthropod diversity and ecosystem functions differ between native forest and exotic tree plantations?
3. Do positive spill over effects of diversity and ecosystem functions exist from forest interior into the agricultural landscape?
4. What can we derive from our results for practical forest conservation and landscape management?

MATERIALS AND METHODS

Study Area

The Taita Hills represent the northern most section of the Eastern Arc Mountain range (Newmark, 1998; Burgess et al., 2007) and host a large variety of endemic (endangered) plant and animal species (Bytebier, 2001). Therefore, the Taita Hills are part of the Eastern Afromontane Biodiversity hotspot (Mittermeier et al., 2011). Pre-colonial human settling started the transformation of natural cloud forest into agroecosystems for smallholder farming (Hohenthal, 2018), and forest replacement by exotic tree plantations (Teucher et al., 2020). This transformation of ecosystems also affects species diversity until today (Mulwa et al., 2021) and species composition (Schmitt et al., 2020). These changes of ecosystems also impact various ecosystem functions which are important for the resilience and recovering of ecosystems, and human livelihood quality. Studies showed that the expansion of eucalyptus trees caused the drying out of major parts of Taita Hills, and subsequently the vanishing of most springs (Hohenthal et al., 2015; unpublished data). That is problematic as water is one of the key pre-requisites for successful smallholder farming. We assume that other ecosystem functions have been negatively affected by these fundamental changes of ecosystems.

Study Set-Up

For the measuring of ecosystem functions, we applied a standardized, low-tech, and easy-repeatable technique – the REFA (Meyer et al., 2015). We measured pollination activity and predation rates. These proxies are of high relevance to conduct successful smallholder farming (Tscharntke et al., 2012). Data collection was performed at the end of the dry season (3–24 September 2021). One study plot (10 × 10 m) consisted of three colored pan traps (yellow, blue, and white) set in the plot center, and 10 artificial caterpillar dummies out of green plasticine (spread across the study plot, with 10 cm minimum distance between single dummies, fixed on a small brown paper pad). In total we established 160 study plots along gradients from dense cloud forest interior into open agroecosystems, and additional 20 plots within exotic plantations. Gradients were set perpendicularly to the border of the forest. For each gradient, we established two study plots inside the forest (8 m and about 200 m distant from forest edge), one plot at the forest border (0 m), and five study plots across the agricultural land (with 8, +16, +32, +64, +128 m distance from the previous study plot, following a logarithmic scale). The aim of integrating gradients in our study was to test for potential spillover effects of ecosystems functions from natural forest into the adjoining agroecosystems (see section "Introduction"). In parallel, we used these data to compare levels of ecosystem functions for the different habitats. For this comparative approach we used data from the following habitat types and study plots: Cloud forest (200 m distance from forest edge, $N = 20$ plots), agricultural land (248 m distance from forest edge, $N = 20$ plots), forest border ($N = 20$ plots), and eucalyptus tree plantations ($N = 20$ plots). The exact location of each study plot was measured with a handheld GPS Device (Garmin GPSMAP 64s), and visualized in **Figure 1**. In the following, we describe the two proxies of ecosystem function assessed based on the REFA method, and the collection of arthropods in tree canopies using flight interception traps.

Rapid Ecosystem Function Assessment

Pollination activity is crucial for successful agriculture and high crop yields (Klein et al., 2008). We measured pollination activity based on the total number of insects caught with yellow, white and blue pan traps. Several studies have shown that these colors exhibit highest sampling efficiencies across a wide array of different taxa of flying insects (Campbell and Hanula, 2007; Westphal et al., 2008; Wilson et al., 2008; Nuttman et al., 2011). Pan traps were filled with saturated salt solution with dish washer and placed at a height of about one meter above ground level. Three traps were positioned in the center of each study plot. The pan traps were activated for a total time span of 7 h, from morning till late afternoon. We subsequently sorted the material according to orders Hymenoptera (excluding Formicidae), Coleoptera, Diptera, and Lepidoptera. In addition, we considered representatives of the family Formicidae.

Natural pest control may significantly increase agricultural yields. Therefore, we measured the level of pest control by measuring predation rates. For this, we counted attacks on artificial caterpillars made out of green plasticine (Loiselle and Farji-Brener, 2002; Koh and Menge, 2005; Ruiz-Guerra et al., 2012). This method allows to differentiate among predator groups (in our case we differentiated among insects, birds, rodents, and snails) by respective bite marks on the green plasticine (cf. Howe et al., 2009). We used 10 × 2 cm long caterpillar dummies at each study plot. The caterpillars were exposed for 24 h to attract both, diurnal as well as nocturnal predators. Subsequently, we assessed all bite marks and calculated the proportion of dummies with at least one bite mark. As one predator may cause several bite marks, we did not consider bite frequency per dummy. Thus, more than one bite mark per species would be recorded as 1 record per dummy. Vanished dummies were classified as predated, without any further information on the group of predator (cf. Meyer et al., 2017).

Flight Interception Traps in Tree Canopies

Apart from the two proxies of ecosystem functions, we also assessed arthropod diversity in canopies of trees, across all four habitat types. For data collection, we used light traps equipped with blue, green, and UV LEDs, which were attached to four panes (10 × 30 cm each) of acrylic glass. A collecting jar underneath was filled with 70% Ethanol. A power bank provided electricity for two nights (>48 h). We installed light traps in the lower canopies about 4 m above ground. Sampling was done at five locations for each of the four habitat types (inside the cloud forest Chawia, along the forest edge of Chawia bordering open agricultural land, open agricultural land, and plantations of exotic eucalyptus trees, in total 20 sampling sites). Arthropod sampling was repeatedly conducted for 2 days and nights at each sampling site. Each trap was activated the whole 48 h to utilize the passive effect of the flight interception traps during the day, and the attractive effect during night. We emptied the traps every second day at 5 p.m., changed the batteries and relocated them. Each trap was set at least 1 h before sunset. Sampling was conducted during a 10-day sampling campaign (13-23 September 2021). Some of the UV LEDs inside the Chawia cloud forest were placed in the dense leaf canopy. Therefore, the radiant power of the LEDs was limited in space. Distances among traps were at least 150 m, and the UV LEDs were placed so that the light of the neighboring lamp was not visible to reduce spatial autocorrelation (see Horak, 2013).

Environmental Parameters

Local environmental conditions might impact biodiversity and ecosystem functions. Thus, we measured the following environmental parameters for single study plots: Estimated cover of leaf litter (no, little, medium, and high); estimated cover of herbs, shrubs, and canopy-closure (all with this scale 0–25, 25–50, 50–75, 75–100%); estimated flower availability (no, little, medium, and high).

Statistics

We used generalized linear modeling (GLM) to link pollination activity, predation rates, and biodiversity measures (response

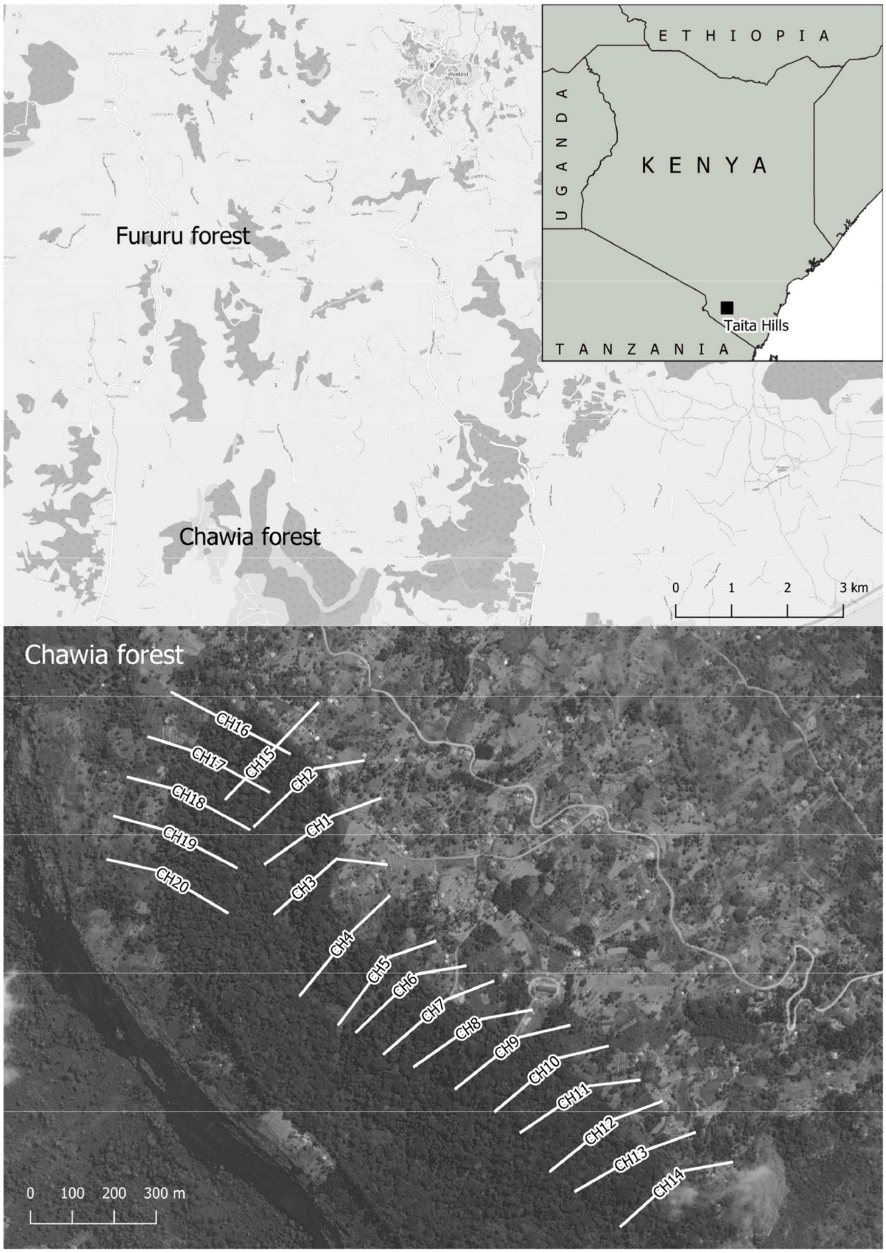

FIGURE 1 | The Taita Hills study area in Kenya (small inlet map), with the two forest patches, Fururu forest (plantation) and Chawia cloud forest, surrounded by open agricultural land. Gradients are shown in the large map below.

variables) to the above mentioned environmental parameters (categorical predictors), and to the distance from forest edge (metric parameter). As the response variables were based on count data, we used a Poisson error structure and a linear link function (O'Hara and Kotze, 2010; Little, 2013). Single effects of habitat structure were assessed from one-way ANOVA and *post hoc* Tukey tests. We estimated the degree of habitat variability from the coefficient of variation CV = σ/μ of the two REFA variables within each habitat type, where σ and μ are the SD and the mean variable expressions. Calculations were done with Statistics 12.0. Errors refer to standard errors and bootstrapped confidence limits.

RESULTS

The four habitat types (cloud forest, forest edge, agricultural land, and exotic tree plantations) differed with respect to predation rate and pollination activity (**Table 1** and **Figure 2**). Predation rate was highest in the cloud forest and pollination activity was highest at the forest edge (**Figure 2**). Pollination activity, i.e.,

TABLE 1 | Average values of major pollinator abundances (blue, yellow, and white traps combined) in the four habitat types.

Habitat	Hymenoptera	Coleoptera	Diptera
Agriculture	**1.00 ± 0.24**	1.09 ± 0.46	**3.57 ± 0.54**
Forest edge	**0.89 ± 0.26**	0.37 ± 0.14	**3.11 ± 0.63**
Forest	**0.74 ± 0.21**	0.53 ± 0.23	**1.68 ± 0.41**
Plantation	**0.35 ± 0.13**	0.15 ± 0.08	**1.35 ± 0.31**

Errors denote standard errors. Bold types mark significant differences in post hoc Tukey comparisons after one-way ANOVA at $P(F_{3,176}) < 0.05$.

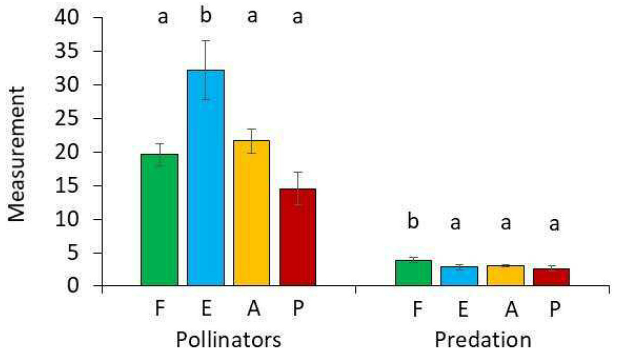

FIGURE 2 | Average numbers of pollinators (all color traps combined) and bite marks (arthropod predation) in the forest (F, green), the forest edge (E, blue), the agricultural fields (A, orange), and the tree plantation (P, red). Different letters above the bars denote statistically significant differences in pair-wise U-tests after one-way Kruskal–Wallis ANOVA at $P < 0.05$. Error bars denote standard errors.

Hymenoptera, Coleoptera, and Diptera abundances were lowest in the plantations and highest in the agricultural land (**Table 1**). Trapping with flight-interception traps in canopies returned very similar results (**Figure 3**). Forest edges and agricultural land were richest in total insect numbers (**Figure 3A**) and major insect orders (**Figure 3B**). The plantations were in both cases devoid of insects (**Figure 3**). The GLM returned significant differences in pollination activity with respect to habitat characteristics (**Table 2**). Pollination activity was lowest in places without herb cover (**Figure 4A**) and low flower numbers (**Figure 4D**). Predation rates increased with increased shrub cover (**Figure 4C**). Leaf litter cover did not significantly influence predation and pollination activity (**Figure 4B**).

We further looked at the variability of the measured function within each habitat type (**Figure 5**). We found relatively low coefficients of variation of CV < 1 indicating a lower within habitat variability than expected from a Poisson distribution. For the two measures (pollination and predation), variability was highest within the cloud forest and lowest in the exotic tree plantations (**Figure 5**). All raw data (pollinator activity, predation rate, flight interception traps, and environmental parameters) are given in **Supplementary Appendix 1–4**.

Finally we tested for significant spill over effects of ecosystem functions, from the forest into the open agricultural lands. Plots of numbers of insect bites in the dummy larvae (**Figure 6A**) and numbers of individuals of flying arthropods in pan traps revealed a marked although statistically insignificant trend [ANOVA: $P(F_{8,171}) > 0.1$] of increased bites and Hymenoptera individuals 8 and 16 m inside the agricultural land, and higher predation rates inside the forest than in open agroecosystems. Numbers of Diptera peaked around the forest edge while Coleoptera numbers increased toward the agricultural land (**Figure 6B**).

DISCUSSION

We found a trend toward higher predation rates in heterogeneous and natural cloud forests than in the surrounding agroecosystems (with lowest rates in tree plantations). Bite marks were mainly from insects (ants), but also from small mammals (exclusively inside the forest). We conclude that the abundance of arthropods (mainly ants) is significantly higher inside natural forest than across the disturbed agricultural landscape. The obtained higher level of predation inside of forest indicate a potential source for spill over into the adjoining agroecosystems. This phenomenon was already shown in previous studies (Klein et al., 2003, 2007; Ricketts, 2004; Tscharntke et al., 2008; Calvet-Mir et al., 2012;

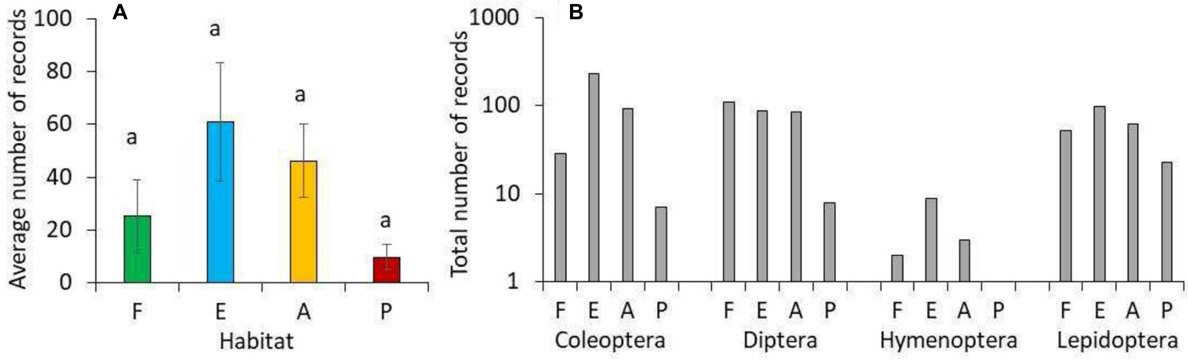

FIGURE 3 | (A) Average numbers of insects recorded in light traps in the forest (F, green), the forest edge (E, blue), the agricultural fields (A, orange), and the tree plantation (P, red). Identical letters in **(A)** above the error bars indicate lack of statistically significant differences in pair-wise U-tests after one-way Kruskal–Wallis ANOVA at $P < 0.05$. Error bars denote standard errors. **(B)** Respective numbers of four major insect orders.

TABLE 2 | Generalized linear modeling (Poisson error structure, linear link function) detected significant influences of important categorical habitat structures (leaf litter, herb, and shrub cover) on pollinator abundances but not on predation pressure (number of bite marks).

Variable	df	Pollinators	Predation	Hymenoptera	Coleoptera	Diptera
Leaf litter	3	14.53**	4.64	24.***	24.***	55.7***
Herb cover	4	26.99***	2.52	12.8*	12.8*	13.2*
Shrub cover	6	15.50*	8.29	31.4***	31.4***	84.3***
N_{flower}	3	4.28	3.31	1.11	1.11	50.9***
Δ_{FE}	1	5.45*	6.06*	5.6*	5.6*	12.8*
Δ_{FE}^2	1	0.05	0.03	3.31	3.31	72.3***

Z-transformed distances (Δ_{FE}) and squared distances (Δ_{FE}^2) to the forest edge served as covariates. Given are Wald values. N = 180.
*Parametric significances: *P < 0.05, **P < 0.01, ***P < 0.001.*

Habel and Ulrich, 2020) and interpreted as a positive effect on agricultural productivity. Predation may support the regulation of potential pest outbreaks, and thus is of high relevance for smallholder farmers and food-security (cf. Olfert et al., 2002; Haddad et al., 2009). Therefore, it has been increasingly recommended that small forest islands remain during forest clearings, to guarantee a minimum of ecological pest control function, and thus to preserve the resilience of landscapes (Oliver et al., 2015). We found no trend of potential spill over of pollinator activity from forest into agroecosystems, but higher pollination activity throughout the agricultural landscape. Most pollinators depend on ample sunlight, and are therefore found in open landscapes with an adequate supply of flowers (see below). This is the case in the extensively and heterogeneous agricultural landscapes of Taita Hills, consisting of small fields, fallow land, and gardens around housings. These results underline that the co-existence of various ecosystems accelerate ecosystem functions, as long as farming remains rather is not too intense (Lichtenberg et al., 2017), as long as the cultivation of the fields is largely extensive and small-scale.

Ecosystem Functions in Different Ecosystems

Our data show that arthropod diversity and ecosystem functions vary among different types of ecosystems, and show partly contradictory trends. Previous studies showed that different ecosystems provide different habitat structures and resources, and thus are home of various plant and animal species, which subsequently provide ecosystem functions (Weisser et al., 2017). Arthropod activity such as pollinator activity is strongly influenced by solar radiation, and therefore pollination activity is greatly reduced in shaded and dense forest, but increases abruptly at the forest edge, as shown by our data (pan-traps and flight interception traps). This trend goes in line with other studies showing that flying arthropods accumulate particularly at sunny and flower-rich spots, which are more frequently found in open agroecosystems (Nicholls and Altieri, 2013; Isbell et al., 2017), as well as along ecotones (here the transition from forest into agroecosystem). We found that predation rates are comparatively high inside the forest. This finding goes in line with results from

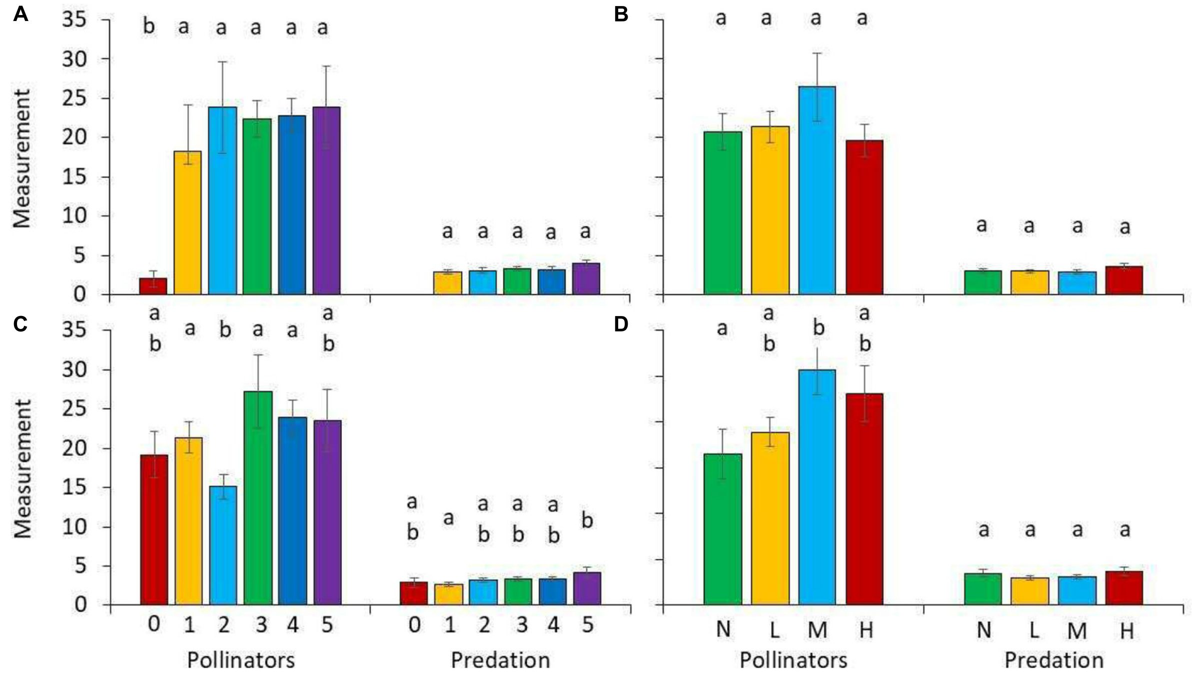

FIGURE 4 | Average numbers of pollinator activity (all color traps combined) and arthropod predation (bite marks) in dependence on the fraction of **(A)** herb cover, **(B)** leaf litter, **(C)** shrub cover (0: 0%, 1: 1–24%, 2: 25–49%, 3: 50–74%, 4: 75–99%, and 5: 100%), and **(D)** number of blossoms (**n**one, **l**ow, **m**edium, **h**igh). Different letters above the bars denote statistically significant differences in pair-wise U-tests after one-way Kruskal–Wallis ANOVA at $P < 0.05$. Error bars denote standard errors.

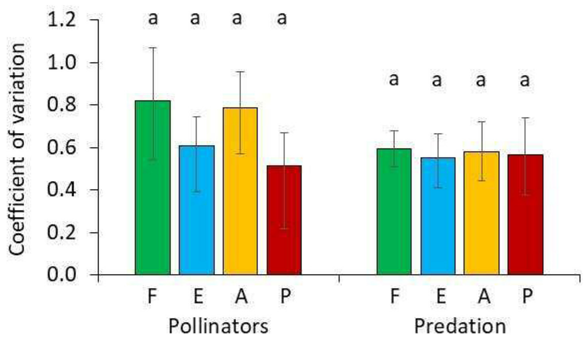

FIGURE 5 | Coefficients of the variability of pollinator activity (all color traps combined) and arthropod predation (bite marks) in the forest (F, green), the forest edge (E, blue), the agricultural fields (A, orange), and the tree plantation (P, red). Identical letters in above the bars indicate lack of statistically significant differences in pair-wise U-tests after one-way Kruskal–Wallis ANOVA at $P < 0.05$. Error bars denote bootstrapped 95% confidence limits.

other studies (Schwab et al., 2021), indicating key preconditions such as microhabitat structures and microclimate accelerating the abundance of arthropods (Schwab et al., 2021). Ground-dwelling arthropods are adversely negatively affected by frequent disturbances through farming activities and thus may build up lower abundances in agroecosystems (Smith et al., 2008; Pardon et al., 2019), which might be a reason of reduced bite marks of dummy caterpillars in our study.

Our data show that eucalyptus plantations provide lowest levels of biodiversity and ecosystem functions. The studied eucalyptus plantations are extremely homogeneous and of only little value to most native species. Thus, plantations of exotic eucalyptus represent only little basis for the development of most organisms (e.g. herbivorous arthropods, Zahn et al., 2009), and subsequently for other organisms at higher trophic levels, such as birds (Mulwa et al., 2021), and thus provide only little ecosystem functions (Pejchar and Mooney, 2009), as approved by our own data. Our results go in line with studies measuring the same proxies of ecosystem functions using the same techniques. The invasion of exotic plant species following ecosystem disturbance resulted in the homogenization of a once heterogeneous and species rich vegetation (Habel and Ulrich, 2021). Here, the spread of the exotic invasive *Lantana camara* shrub species along rivers in south-eastern Kenya resulted in collapse of biodiversity across all trophic levels (Habel et al., 2018), and negatively affect ecosystem functions (Habel and Ulrich, 2021). Similarly, in the case of Taita Hills, intense planting of eucalyptus trees caused shifts in abiotic conditions (such as hydrology and pH-value, see Bewket and Sterk, 2005; Chanie et al., 2013; Guzha et al., 2018), which may fundamentally modified species community structures (John and Kabigumila, 2007; Gardner et al., 2008; Martello et al., 2018; Allingham, 2020). Our results from the Taita Hills show that most plantations (especially when they consist of alien species) can result in drastic species loss (John and Kabigumila, 2007; Gardner et al., 2008), and severe reduction of ecosystem functions (Lemenih and Bongers, 2010; Omoro et al., 2010; Bayle, 2019). In consequence, the plantation of eucalyptus in Taita Hills does not represent suitable surrogate habitats for native plant and animal species.

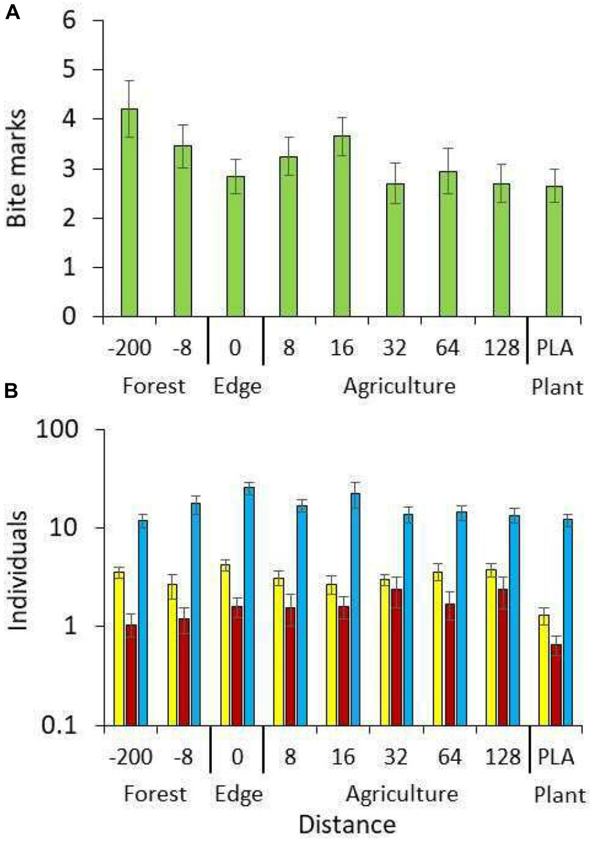

FIGURE 6 | Mean numbers of dummy bitemarks **(A)** and individuals caught with pan traps (**B:** yellow: Hymenoptera, brown: Coleoptera, blue: Diptera) in dependence on distance to the forest. Plantation data (Plant) are shown separately. Error bars denote standard errors.

Local Environmental Parameters and Ecosystem Functions

Our results show that local environmental conditions can influence and significantly increase local environmental functions. For example, pollination activity is positively influenced by the presence of vegetation and flowers. Some studies have already confirmed that landscape heterogeneity and the existence of flower rich sites and fallow land enhance ecosystem functions (cf. Isbell et al., 2017). In consequence, planting flowering plants (e.g., flowering areas and flowering strips) in agricultural landscapes is now a widely accepted tool to maintain and accelerate ecosystem function (e.g., pollination) which subsequently maximize crop yields (Garibaldi et al., 2014; Albrecht et al., 2020). In addition to attracting pollinators by offering flowers, it is necessary for numerous arthropods (including representatives from the groups of Hymenoptera and Diptera) to create fallow land for numerous arthropods with appropriate microhabitat structures (vegetation, bare soil) to support successful larval, i.e., species development.

From Theory to Practice

The results obtained and the correlations show evidence that the co-existence of various ecosystems represent different levels of biodiversity and provide different rates of ecosystem functions, and thus enhance the total value of ecosystem functions at the landscape level. Biodiversity and ecosystem functions can be specifically promoted by targeted ecological upgrading of small areas located in between agricultural land. Seeding flowering areas and flowering strips increases the attractiveness of landscapes, and the presence of pollinators – and thus may accelerate agricultural yields (Albrecht et al., 2020). In addition, the establishment of fallows (resting areas) as potential nurseries for numerous arthropods is essential for agroecosystems to be able to develop at all. The cultivation of exotic and invasive plant species (such as eucalyptus) do not represent a surrogate habitat for most forest specialist taxa and thus cause significant reductions in biodiversity and ecosystem functions, and the devastation of entire landscapes.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by NACOSTI.

AUTHOR CONTRIBUTIONS

JH and MT developed ideas and the study set-up. TS, FM, FG, MT, and JH collected the data. WU performed statistical analyses. All authors contributed while data interpretation and writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.816163/full#supplementary-material

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Seasonal Changes in an Afromontane Forest Bird Community in Tanzania

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Seasonal variation in the composition of avian communities is poorly documented in African montane forests. Using field observations, mist-netting data (63,424 NMH), recording of mixed-species flocks (160 flocks noted), and of the feeding ecology of greenbuls (757 observations), we document marked seasonal changes in a bird community in an East African montane forest (1,340–2,130 m) in the Udzungwa Mountains in south-central Tanzania. This mountain range contains the largest remaining forests in the Eastern Arc Mountains in Tanzania, part of a key global biodiversity hotspot. Our analysis is based on data in the dry and wet season from about 12 months of fieldwork. Field observation data combined with mist-net data demonstrate noticeable seasonal changes in certain species' abundances, indicating (i) seasonal movements out of the montane forest during the dry season and (ii) movements of part of the populations for other species. Our mist-net results show a significant difference in species abundances between the two seasons driven by 16 species. We also document significant changes in diet for two species of greenbuls, which shift feeding behavior from arthropods in the wet season to include a larger proportion of fruit in their dry season diet. Our results further show that birds are more active in mixed-species flocks in the dry season, with a significantly higher average number of species and of individuals in the dry season, i.e., 11.3 (±0.52 SE) species, 32.3 (±1.76 SE) individuals] compared to the wet season 9.7 (±0.78 SE) species, 20.8 (±1.85 SE) individuals]. One of two very distinctive types of mixed-species flocks – confined to the understory – exists only in the dry season. We discuss these changes to seasonal variability in climate, i.e., temperature and precipitation.

Keywords: altitudinal movement, Eastern Arc, mixed-species flocks, seasonality, Udzungwas

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INTRODUCTION

Tropical mountains are centers of global biodiversity, endemism and threatened species (McCain and Colwell, 2011), with rich aggregations of small-ranged species that form centers of endemism (Rahbek et al., 2019a), and contribute disproportionately to the terrestrial biodiversity of Earth, especially in the tropics (Rahbek et al., 2019b). At the same time, the role of mountains as refugia for biodiversity is under serious human threat (IPBES, 2019; Rahbek et al., 2019b).

Climate change is accelerating (IPCC, 2021) and is considered one of the most important drivers of biodiversity loss after changes in land use (IPBES, 2019). Fluctuations in climate conditions are expected to intensify further due to increasing temperatures (IPCC, 2021). Coupled with the high rates of deforestation, forest fragmentation, and degradation in tropical regions (Laurance et al., 2011), biodiversity is severely threatened (Vieira et al., 2008; Eglington and Pearce-Higgins, 2012). This is particularly so in Africa, where deforestation rates are among the highest in the tropics (Lambina et al., 2001; Boko et al., 2007; IPBES, 2018).

On the mountains' elevational gradients, projections of precipitation changes and rising temperature have been shown to have the potential of jeopardizing bird and mammal populations (McCain and Colwell, 2011). Especially, increased drying conditions seem to be developing in areas of Africa (Oguntunde et al., 2017). Additionally, elevational gradients present opportunities for species currently living near their upper thermal limits to track cooler temperatures upslope in warming climates, but only if changes in precipitation are sufficiently in step with temperature (McCain and Colwell, 2011). Temperate species generally have broad thermal tolerances because they are adapted to a more variable climate compared to tropical species (Khaliq et al., 2014), whereas in the tropics, montane species tend to have narrow tolerances, and therefore, montane species are expected to be particularly vulnerable to climate change (Araújo and Rahbek, 2006; Forero-Medina et al., 2011; Laurance et al., 2011; Santillan et al., 2018).

Species will differ in their sensitivity to climate and land-use change, depending on their niche breadth as it relates to habitat and diet (Carscadden et al., 2020). Tropical montane species often have narrow niches adapted to pockets of suitable habitats. Habitat specialists and insectivorous bird species will be more sensitive to disturbances than habitat generalists. The latter will have a greater chance of surviving and thriving in a wider variety of disturbed or seasonally variable habitats (Mulwa et al., 2012; Reif et al., 2013; Powell et al., 2015).

Seasonal changes in the structure of tropical and subtropical species communities have been known for decades (e.g., Karr, 1976), and temporal fluctuations in species abundance are widespread (e.g., Loiselle and Blake, 1991; Malizia, 2001), caused, e.g., by abiotic factors such as rainfall and temperature resulting in changes in food availability and thermal conditions. In particular, rainfall seasonality may result in a bottleneck of resources (insects, nectar, and fruit) during the dry season, which limits the population size of many species (Williams and Middleton, 2008). In Afrotropical climates with pronounced and severe dry/wet seasonal differences, anomalies may be expected to greatly affect the ecosystems (Parmesan and Yohe, 2003; Root et al., 2003), including the composition and dynamics of forest bird communities (see also Pounds et al., 1999). An increasing dryness and disruption of food resources may have a higher impact at higher elevations, where the temperature is lower and therefore influences, e.g., the likelihood of a low abundance of a given bird species. This could disrupt the connectedness among and between species and lead to a reformation of species communities and to extirpations (Root et al., 2003).

Here, we document the seasonal changes in an Afromontane forest bird community from the Ndundulu Mountains. In the Udzungwa Mountains, a global biodiversity hotspot in Tanzania (Wasser and Lovett, 1993; Myers et al., 2000; Dinesen et al., 2001). Based on three simultaneous studies from 1991 to 1992, we provide evidence of changes in community composition, abundance, and behavior between a cold and pronounced dry season and a warm, humid season. Each of the three simultaneous studies were designed to increase the likelihood of detecting seasonal changes by visiting the same forest sites in those two different seasons but focusing on three independent elements of the forest bird community. These are the understorey community as surveyed by mist-netting, as well as feeding behavior of the abundant greenbuls and composition of mixed-species flocks (MSFs) by direct observation. Additionally, systematic general bird observations were collected during extensive walks through the forest in-between MSF observations.

These different sets of forest bird data are investigated through the following null hypotheses: (1) There are no seasonal changes in bird species abundance, (2) there are no changes in diet between seasons, and (3) there are no differences in participation and abundance of forest bird species in mixed-species flocks between seasons. Moreover, we look into different explanations, such as a seasonal change in behavior or movements out of the montane forest.

Study Area

The study was conducted in the Ndundulu Mountains in the Udzungwa Mountains between June 30 and September 9, 1991 (dry season) and between November 5, 1991, and January 9, 1992 (wet season). The study area is situated within what is now Kilombero Nature Reserve at 36°27′ – 36°42′E, 07°39′ – 07°51′S (Dinesen et al., 1993). At the time of fieldwork, the area was classified as West Kilombero Scarp Catchment Forest Reserve (Moyer, 1992, see **Figure 1**). The study area is the western-most part of the largest forest patch on the Udzungwa Plateau with a continuous forest covering of about 161 km^2 (Jensen et al., 2020), including the peak of Luhombero. The entire forest gradient extends from 1,200 to 2,460 m. This part of the Udzungwas is covered by submontane, montane, and upper montane forest, with typical high elevation *Hagenia abyssinica* dominating large areas above 2,100 m. The study area is described in more detail in Dinesen et al. (1993) and Frontier Tanzania (2001).

Flora

"The submontane forest is pristine, with trees often reaching a height of 30 m, some over 40 m or 50 m. The dominant tree is *Parinari excelsa*. Other trees (and shrubs) present include *Bersama abyssinica*, *Cassipourea gummiflua*, *Cola stelecantha*, *Craterospermum longipedunculatum*, *Dicranolepis usambarica*, *Ixora scheffleri*, *Myrianthus* sp., *Oxyanthus speciosus*, *Piper capense*, *Psychotria* sp., *Strombosia scheffleri*, *Strychnos* sp., *Tarenna pavettoides*, *Tarenna quadrangularis*, *Uvariopsis* sp., *Vitex amaniensis*, and *Zanthoxylum gilletii*" (Ehardt and Butynski, 2006).

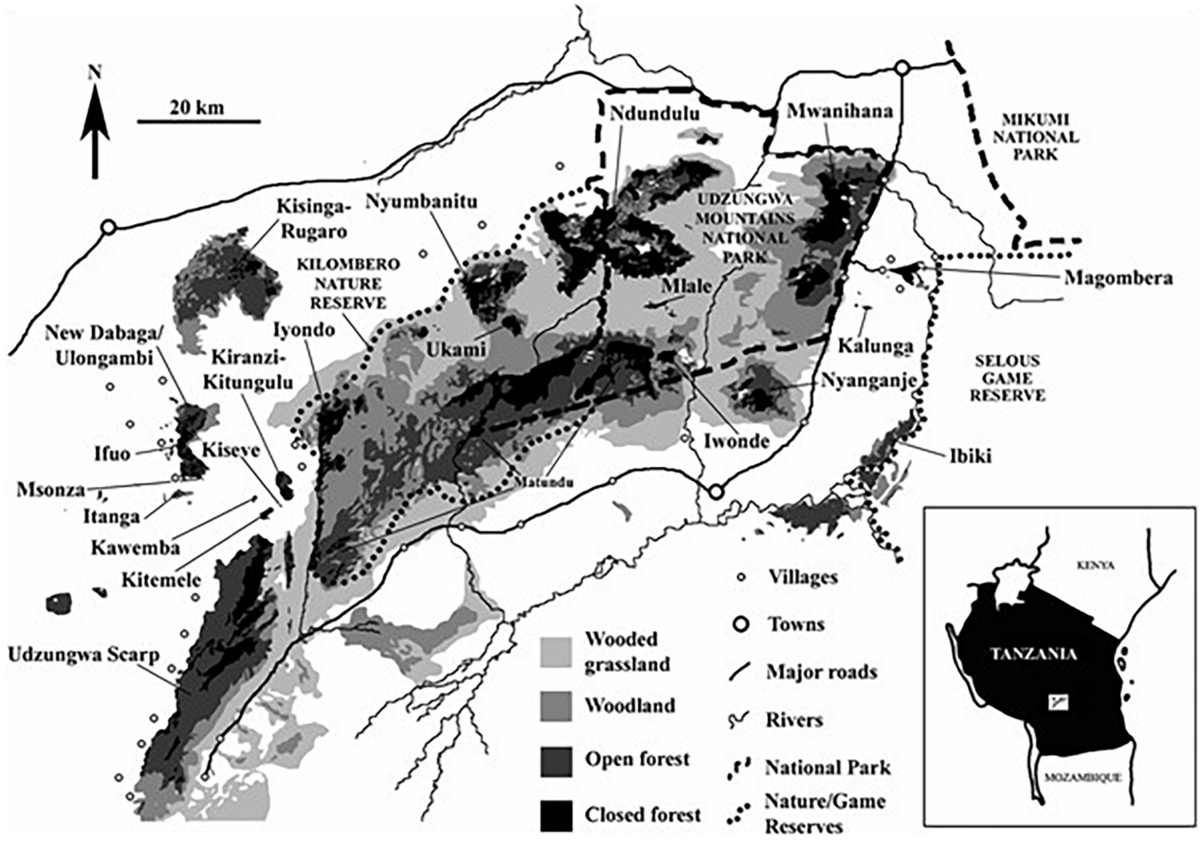

FIGURE 1 | The Udzungwa Mountains in Tanzania. The study was conducted in the Ndundulu Mts. Adopted from Marshall et al. (2009).

Geology and Topography

The chain of ancient gneissic mountains running from the Taita Hills in South-east Kenya to the Udzungwa Mountains in South-central Tanzania has been termed the Eastern Arc Mountains" (Lovett, 1993). They are defined as crystalline mountains under the direct influence of the Indian Ocean climate (Lovett, 1990), and regarded as part of the Tanganyika-Nyasa Mountain Forest Group (Moreau, 1966). As uplifted fault blocks, the mountains appear today as a chain of isolated forest-capped mountains across eastern and south-central Tanzania to the Taita Hills in south-eastern Kenya. Most mountains are habitat islands that rise steeply 1–2 km above the "ocean" of dry savanna plains and harbor a unique flora and fauna, of which many species are endemic to the mountain chain or even to single mountains (Wasser and Lovett, 1993; Burgess et al., 2006).

One of the largest mountain blocks of the Eastern Arc is the Udzungwas, located centrally in Tanzania at the Southwest terminus of the mountain chain. The distance to the Indian Ocean from the Udzungwas varies from about 270 km at Mwanihana (east), and 470 km at Mufindi (west). The length of the Udzungwa Mountains is roughly 240 km from south to north, and the topography varies from foothills and lowlands at c. 300 m to peaks well above 2,000 m.

Climate

Studies of marine sediments from the Indian Ocean reveal that the surface temperature here was only 1–2°C lower during the ice age and did not decline at all off the Tanzanian coast (Prell et al., 1980). This coastal zone may therefore have been permanently warm and humid, and the Eastern Arc Mountains, including the Udzungwas, may therefore have retained patches of humid forest permanently through very long periods (Raven and Axelrod, 1978; Prell et al., 1980). However, more recent studies in the Udzungwas indicate large variations in forest cover within the last 45,000 years and widespread burning (Finch et al., 2014). The high-altitude areas have a climate very different from the foothills as a result of precipitation and temperature changes. Mean annual rainfall in the Udzungwas along the south-eastern scarp is c. 2,000 mm, decreasing to 900 mm on the western plateau (Ehardt et al., 2005). At Mwanihana in the east, with a continuous elevational range of moist forest from 450 to 1,760 m, mean annual rainfall at the foot of the escarpment has been measured at 1,747 mm (at 366 m altitude with 14 years of records) (Lovett, 1996). Rainfall on the escarpment slopes is higher and probably 1,800–2,000 mm per year (Rodgers and Homewood, 1982). However, rainfall and local weather vary considerably between seasons (and years) and the specific localities, and the dry season is often with periods of prolonged drought. The warm and humid wet season generally starts in November, lasting to

May, with on average greater than 100 mm of rain each month (Lovett, 1996); but with yearly changes from this general pattern. Temperatures drop rapidly, with altitude with a lapse rate of about 0.6°C per 100 m (Pocs, 1976). Frost has not been reported from the Udzungwas, but in the other Eastern Arc Mountains at high altitudes (e.g., Pocs, 1976).

MATERIALS AND METHODS

Three of the authors (LH, TL, and LD) collected data used for this paper in both dry and wet seasons during a 4-month period of fieldwork in the Ndundulu Mts. in 1991–1992. Data on the bird community were gathered on a daily basis when the weather permitted it (on occasion rain and fog prevented data collection in the wet season). In general, studies often took advantage of the trails made by African savanna elephants (*Loxodonta africana*) and Cape buffaloes (*Syncerus caffer*), which facilitated walking during surveys. Here follows details on field observations, observations of mixed-species flocks, the diet of greenbuls, mist-netting, and weather data. We also assign each of the montane forest birds into specific feeding guilds comprising seven categories: carnivore, granivore-insectivore, frugivore-insectivore, insectivore, insectivore-frugivore, omnivore, and nectarivore-insectivore (see Annex 1). MSFs turned out to be a common and dominant feature in the forest just like in a wide range of other forested habitats worldwide, birds joining flocks benefit by increased foraging rates and 'safety in numbers' (see e.g., Sridhar et al., 2009). Moreover, greenbuls are among the most abundant species, and the six species involved in the study were highly abundant at certain altitudes, indicating successful feeding strategies. Furthermore, in order to detect changes in overall species composition, including elusive understory birds, mist-netting and general field observations were undertaken. The data collection methods are described in more detail below. We follow the nomenclature of IOC World Bird List (Gill et al., 2021).

Mixed-Species Flocks

We defined an MSF as consisting of two or more species, foraging less than 10 m apart, staying together for at least 5 min, and all members clearly moving together in the same direction, which is more rigid than, e.g., Goodale and Beauchamp (2010). The number of individuals of each participating species was noted. The passage of a flock often caused a burst of activity from other resident (territorial) birds. These could be singing, calling, and/or moving toward the flock. As aroused birds did not start feeding and moving with the flock, they have been excluded from the MSF material and analysis. In general, a flock was followed for as long as it was necessary to count and identify all individuals, resulting in a longer observation time for the large flocks. All MSFs were recorded within forest habitats. The composition of MSFs was recorded on a Dictaphone and transferred to notebooks on a daily basis. Unidentified birds were noted but not used in the analysis. Time and effort were not standardized for the different elevations. Data on MSF was collected by TL with an effort of 55 and 47 field days in the dry and wet seasons, respectively, between 1340 and 2000 m.

For the analysis of the MSF data, we use a generalized linear model (GLM) with a log link function to test for seasonal differences in (a) the number of flocks observed per day, (b) the number of species per flock, and (c) the number of individuals per flock. The GLM assumes that these three response variables follow a Poisson distribution. We then test for seasonal differences in species-level occupancy (presence–absence in flocks) and abundance (number of observed individuals per flock). The occupancy analyses are conducted using a GLM based on a Bernoulli probability distribution and a logit link function. For the abundance analyses, we use a GLM of the previous Poisson-type. To ensure sufficient data for each analysis, we exclude species observed in less than five flocks. Due to the numerous independent tests, we apply Bonferroni correction on the p-values (i.e., multiplying the p-values by the number of conducted tests; $n = 28$). Finally, the seasonal turnover in the mixed flocks' species composition is illustrated using Non-metric Multi-dimensional Scaling (NMDS) from the 'vegan' R package (Oksanen et al., 2019).

Feeding Ecology of the Forest Greenbuls

Data on the following species were collected: Yellow-streaked Greenbul *Phyllastrephus flavostriatus,* Placid Greenbul *Phyllastrephus placidus,* Shelley's Greenbul *Arizelocichla masukuensis*. Stripe-faced Greenbul *Arizelocichla striifacies*, Little Greenbul *Eurillas virens,* and Yellow-throated Greenbul *Arizelocichla chlorigula*. Observations were primarily done in the morning and in the afternoon. All feeding records were written in a notebook with notes on species, date, time, elevation, feeding behavior, diet, height above ground, the canopy height, the number of individuals, and whether the individuals took part in an MSF (see Dinesen, 1995 for details). When a greenbul was sighted, it was followed until the first food item was captured, or an unambiguous substrate search was observed and then used as a feeding record. No further records were obtained of that particular individual. Simultaneously feeding observations of individuals or groups are treated as being independent, even though the feeding behavior of one bird may influence that of another (see Hjejl et al., 1990; Recher and Gebski, 1990; Sherry, 1990). The observations were carried out between 1,340 and 2,000 m in both seasons with an effort of 55 and 40 days, respectively, undertaken by LD. Observation effort was not standardized along the elevational gradient. The classification scheme of foraging behavior proposed by Remsen and Robinson (1990) is followed. However, we only use diet in this analysis.

We use the Chi-squared test to analyze the changes in diet between seasons for the six species of greenbuls. The null hypothesis is that the distribution of food choices remains the same between the seasons. Because there are only two categories in each frequency class, we apply Yates' correction of continuity (Yates, 1934), which involves subtracting 0.5 from the numerator component of the Chi-square formula:

$$\chi^2 = \sum \frac{(|O - E| - 0.5)^2}{E}$$

Here, O and E represent the observed and expected frequency, respectively.

We also investigate shifts in food preferences for each species individually. Due to the low number of registered individuals for some classes generating expected frequencies < 5, we could not use Chi-squared tests. Instead, we applied Fisher's exact test in R (see R Core Team, 2020), which does not have the same requirements for sample size. In the tests for individual species, we apply Bonferroni correction on the p-values to compensate for the increased type I error rate under multiple testing.

Mist-Netting

Data was collected by LAH on the elevational gradient of 1340 to 2130 m in both seasons. Mist-nets were of the brand North Ronaldsay, mesh-size was for passerine birds. The ringing stations were placed near the camp to reduce the handling time of the bagged birds. Mist-nets were erected for every 200 m along a more or less long "straight" line from the lowest camp to the upper camp, and all faced toward the southeast. Each net or group of nets were placed to cover as many different vegetation types as possible, but areas with no undergrowth were avoided as initial netting had shown that very few birds were caught here. Each net was opened shortly before the first song of the dawn chorus and remained open until sunset. We deviated from this standard only due to rain or thick wet fog. Each net was checked at least once per hour and opening and closing time were noted. If weather conditions permitted, each net was erected for at least two full successive days. A mist-net with, e.g., a length of 12 m operated for 12 h has a total NMH (net meter hours) of 144 NMH. Therefore, the rate at which birds are captured in mist-nets provides a "simple" index for comparison for a "mist-net abundance" of the undergrowth birds at different sites within the forest. As a minimum, we sampled each site in both seasons for 2,500 NMH.

We use a Chi-squared test to analyze the community turnover between the dry and wet seasons. This test is sensitive to rare species in the contingency **Supplementary Table S1**. As a rule of thumb, less than 20% of the expected frequencies should be <5, and none should be less than 1 (Fowler et al., 1998). To meet these criteria, we excluded 13 species from the analysis, all singletons ($n = 10$) or doubletons ($n = 3$): African Goshawk *Accipiter tachiro*, Black-headed Apalis *Apalis melanocephala*, Bar-tailed Trogon *Apaloderma vittatum*, Dapple-throat *Arcanator orostruthus*, Black-fronted Bushshrike *Chlorophoneus nigrifrons*, Klaas's Cuckoo *Chrysococcyx klaas*, Olive Woodpecker *Dendropicos griseocephalus*, Collared Sunbird *Hedydipna collaris*, Scaly-throated Honeyguide *Indicator variegatus*, African Dusky Flycatcher *Muscicapa adusta*, Yellow-rumped Tinkerbird *Pogoniulus bilineatus*, Green Barbet *Stactolaema olivacea*, and Tambourine Dove *Turtur tympanistria*. We stepwise removed species and recalculated the Chi-squared test to identify the species contributing most to the seasonal community turnover. The few records of these 13 species can be explained by the fact that a few were low-density species (e.g., Dapple-throat) or occurred in mid-stratum and/or canopy or are species attracted to the forest edge and light gaps, making it less likely for these to be caught by a mist-net.

We apply the Chao 1 estimator (Chao, 1984; Colwell and Coddington, 1994) to estimate the 'true' species diversity in each season.

$$Chao\ 1 = S_{obs} + \frac{F_1^2}{2F_2}$$

Here, S_{obs} is the sampled number of species. F_1 is the number of species encountered once (i.e., singletons). F_2 is the number of species encountered twice (i.e., doubletons).

Field Observation Data

We used 10x40 Zeiss binoculars. We use TL's general field observation data while looking for mixed-species flocks. He noted all birds seen and heard during walks in the forest between 1,340 and 2,000 m on the entire elevation gradient. All individuals encountered were identified and noted with date, altitude, and time period for each field survey. Data were gathered during both the dry and wet season with an effort of 55 and 47 field days, respectively. We assess that TL's field observations (including all bird observations in and between MSFs) provide a realistic picture of the composition of the bird community as the observations covered the different forest strata in the range from 1,340 to 2,000 m and were collected consistently.

Weather Data

A rain gauge (Wilh. Lembrech Göttingen with a capacity of 50 mm) was set up next to our campsite. Readings had an accuracy of 0.1–0.5 mm (the shape of the gauge was conical). All measurements of precipitation are of direct rainfall. The Temperature was measured with an electronic min–max thermometer with an accuracy of ±0.5°C, and manual readings were taken at 0600 h, 1200 h, 1500 h, and 1800 h. Only readings within ±10 min of the hour in question have been used. Most measurements were taken at 1,720 m. Temperature is sensitive to altitude, and measurements at other altitudes have been corrected for altitude and adjusted to this elevation, which has been used as standard. All measurements were taken from a fixed position in the shade at roughly 150 cm above the ground and under rainfall cover. Weather data were collected by LAH during both seasons.

RESULTS

Seasonal Change in Local Weather

Figure 2 shows the marked seasonal change in local weather during our study. The temperature increased about 5°C from an average of 14.5°C to 19.2°C (**Figure 2A**). The minimum temperature recorded was 4°C (August 30 and September 8, 1991) in the dry season, and maximum was 25°C (18 December 1991) in the wet season. There was a marked change in rainfall as well (**Figure 2B**), as almost zero rainfall was recorded (7 mm) in our study area in the dry season, increasing to about 900 mm in the wet season (**Figure 2B**). The dry season was pronounced and long, as we have observed in other years of fieldwork in the same area (LD, TL, and LAH).

We recorded a total of 94 montane forest bird species in the study area, excluding some temperate migrants such as,

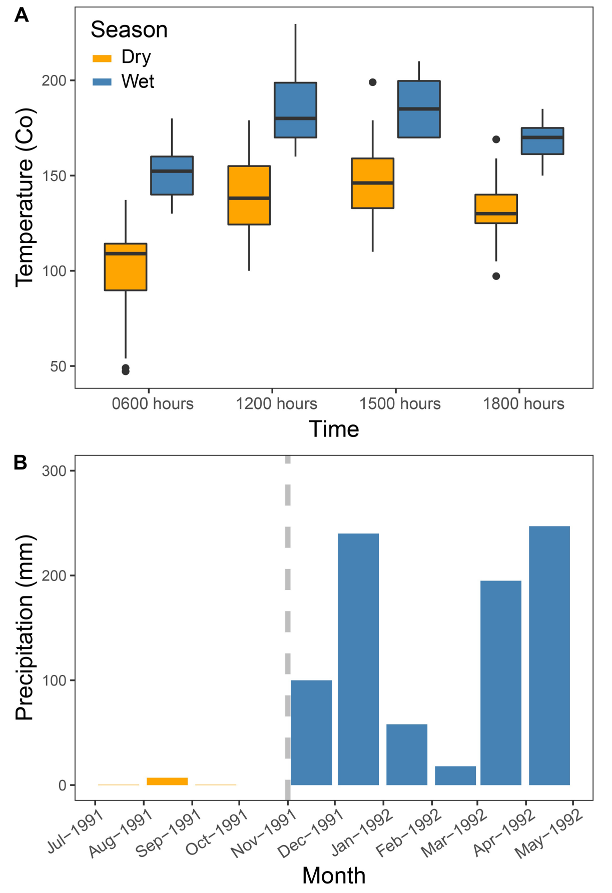

FIGURE 2 | Rainfall and temperature measured at the study site and during the study period from July to August 1991 (dry season) and again from November to January 1992 (wet season) at different camp sites at different altitudes. **(A)** Temperature readings were made every hour between 0600 h and 1800 h. No measurements were taken during October. **(B)** Here, we present four values as averages measured at different campsites at different altitudes in the study area during the period from July to September1991 (dry season) and again from November 1991 to January 1992 (wet season).

e.g., Blackcap *Sylvia borin*, and Thrush Nightingale *Luscinia luscinia* observed at the forest edge. Following Jensen et al. (2020) and including species in MSFs, the bird community comprises 40 (50%) insectivorous species, 17 (21%) mainly frugivore-insectivore (omnivorous) species, nine (11%) insectivore-nectarivore species, five (6.3%) granivorous species as well as six (7.5%) birds of prey (see Annex 1). Based on mist-net data, MSF, and field observation data, we developed species accumulation curves (**Figure 3**). The cumulative number of species increased drastically during the initial sampling period but eventually flattened (**Figures 3A,B**). This pattern indicates that our samples are good representations of the actual species communities, providing confidence in our results.

Almost 50 species were recorded in the mist-nets in the wet season (**Figure 3A**) where the species accumulation curve rise faster than in the dry season. Fewer bird species were caught in the dry season as well as fewer individuals, and the curve is still increasing. In total, 72 species in the dry and 74 in the wet season were recorded in the MSF and field observation data (**Figure 3B**) with a faster accumulation in the wet season as well. Thus, the graphs indicate that the speed of adding new species was slightly slower during the dry season; however, it should be noted that we were more familiar with the species identification in the wet season as it comprised our second field period. The two species accumulation curves (**Figures 3A,B**) should not be compared with each other because mist-netting was conducted over a longer gradient at the upper end than the MSF and the field observation data, and therefore mist-netting data includes species that was not encountered at lower elevation. The overall picture is that both species numbers and abundance are higher in the field observation data (including MSFs) because this method includes records of birds from all forest strata.

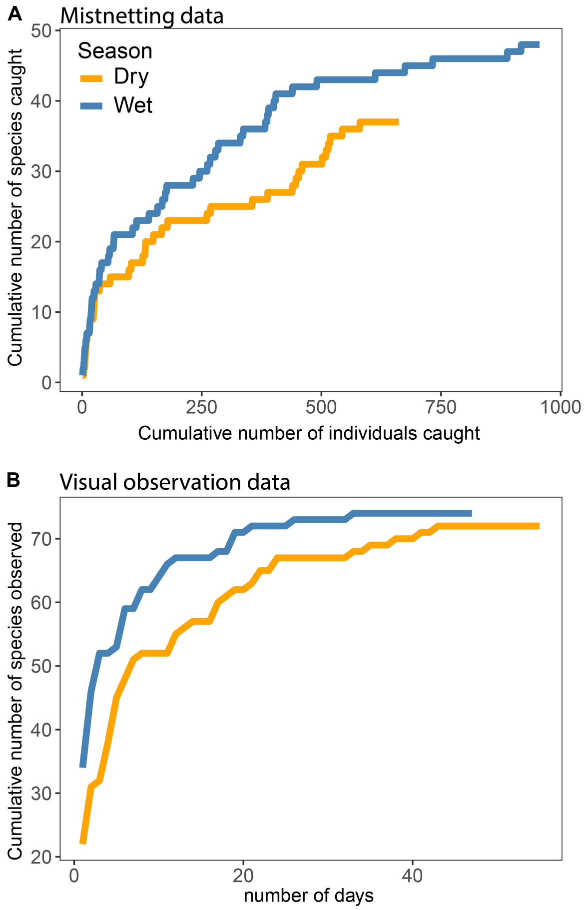

FIGURE 3 | Species accumulation curves based on mist-net **(A)** and on field data **(B)**.

An additional number of species were recorded inside the forest during TL's collection of field observation data, none of which were treated in Jensen et al. (2020) and also not further analyzed in this paper. None of these participated in the MSF's, but all were recorded at altitudes from 1340 m and upward, and may satisfy the criteria set in Jensen et al. (2020)

for qualifying as a montane forest species (>1200 m): Crowned Hornbill *Lophoceros alboterminatus* (common, both seasons, whole gradient), Tambourine Dove (>5 observations), Crested Guineafowl *Guttera pucherani* (once, dry season only, 1700 m) and Green-backed Honeybird *Prodotiscus zambesiae*. The following species were also recorded inside the forest, but are considered as forest edge species in our case and/or have other habitat preferences: African Black Duck *Anas sparsa*, African Green Pigeon *Treron calves*, Little Sparrowhawk *Accipiter minullus*, Black Sparrowhawk *Accipiter melanoleucus*, White-necked Raven *Corvus albicollis*, White-eyed Slaty Flycatcher *Melaenornis fischeri*, Ashy Flycatcher *Muscicapa caerulescens*, Yellow-bellied Waxbill *Coccopygia quartinia*, Lesser Seedcracker *Pyrenestes minor*.

Mixed Species Flocks

During the dry season, a total of 111 mixed-species flocks were recorded during 55 field days. Of these, 96 fulfilled the criteria of at least 95% of all individuals identified. This slightly reduced data set was used for analysis, with a total of 50 species identified as participating in the MSFs. During the wet season, 49 MSFs were recorded, with a total of 37 species in 47 field days. One distinct type of MSF (labeled canopy MSF) was generally confined to the higher mid-stratum and canopy and was recorded both during the dry ($n = 42$) and the wet ($n = 40$) season. The canopy MSF comprised the following species (n = the number of flocks attended): Yellow-streaked Greenbul ($n = 81$), Common Square-tailed Drongo *Dicrurus ludwigii* ($n = 78$), Dark-backed Weaver *Ploceus bicolor* ($n = 77$), Black-headed Apalis ($n = 74$), Yellow-throated Woodland Warbler *Phylloscopus ruficapilla* ($n = 46$), Brown-headed Apalis *Apalis alticola* ($n = 44$), Grey Cuckooshrike *Ceblepyris caesius* ($n = 44$), Black-fronted Bushshrike ($n = 38$), Olive Woodpecker ($n = 37$), Shelley's Greenbul ($n = 37$), Southern Yellow White-eye *Zosterops anderssoni* ($n = 29$), Green Barbet ($n = 28$), Stripe-faced Greenbul ($n = 24$), Moustached Tinkerbird *Pogoniulus leucomystax* ($n = 21$), White-winged Apalis *Apalis chariessa* ($n = 20$), and Chapin's Apalis *Apalis chapini* ($n = 18$). An additional 34 species were attending 15 or fewer canopy MSFs.

The only other distinct type of MSF was confined to the understorey (labeled understorey MSF), but was only encountered during the dry season ($n = 16$). The understorey MSF's were mainly composed of the following species, where n = the number of flocks attended: Placid Greenbul *Phyllastrephus placidus* ($n = 16$), Dark Batis *Batis crypta* ($n = 12$), Shelley's Greenbul *Arizelocichla masukuensis* ($n = 9$), Yellow-throated Woodland Warbler ($n = 9$), Bar-throated Apalis *Apalis thoracica* ($n = 7$), Red-capped Forest Warbler *Artisornis metopias* ($n = 5$), White-tailed Crested Flycatcher *Elminia albonotata* ($n = 5$), Fülleborn's Boubou *Laniarius fuelleborni* ($n = 4$), and African Hill Babbler *Sylvia abyssinica* ($n = 4$). An additional 12 species were attending three or fewer understorey MSF's. A total of 38 flocks in the dry season and nine flocks in the wet season could not be assigned to any of the above two distinct types/categories of MSFs and were disregarded in the analysis (see Annex 2, sheet '**Table 1** and **Figure 4**,' where the 38 and 9 flocks are marked with '3').

The average number of MSFs observed per day in the dry season was 2.02 (±0.13 SE) and 1.04 (±0.18 SE) during the wet season. There were two field days in the dry season with zero flocks and 15 days with zero flocks in the wet season. Using a Poisson GLM with a log link function, we found significantly more flocks per day during the dry season than in the wet season. $\beta = 0.66$, $SE = 0.17$, p-value < 0.001.

The average number of species in the canopy MSFs was 9.7 (±0.78 SE) in the wet season, and 11.3 (±0.52 SE) in the dry season (standardized coefficient (β) = 0.15, Standard error (SE) = 0.07, p-value = 0.025). Hence, there were significantly more species per flock in the dry season. There were also significantly more individuals per flock during the dry season, with an average number of individuals of 20.8 (1.85 SE) in the wet season, compared to 32.3 (±1.76 SE) in the dry season ($\beta = 0.43$, $SE = 0.02$, p-value < 0.001).

A total of 50 species were recorded in the canopy MSFs; however, only 36 species were observed in more than five flocks. We used five occurrences to fulfill the statistical analysis criteria. Among these 36 species, only two species showed significant differences in occupancy between the two seasons. Brown-headed Apalis and Yellow-throated Woodland Warbler occupied significantly more flocks during the dry season than during the wet season (see **Table 1**). In the analysis testing for seasonal differences in the species' abundance, only Yellow-streaked Greenbul turned out as significantly more abundant in the dry season ($\beta = 0.73$, $SE = 0.11$, corrected p-value < 0.001). Lastly, we visually compared the canopy MSFs between seasons using an NMDS plot (**Figure 4**), which incorporates both the turnover in species composition and abundances. Here, it becomes apparent that only three flocks are compositionally similar between the wet and the dry season and hence a clear difference in composition between the dry and the wet season canopy MSFs.

Feeding Ecology of Greenbuls

Six species of forest greenbuls were present within the study area. All-together 757 feeding observations (320 from the wet and 430 from the dry seasons) were recorded: Shelley's Greenbul ($n = 192$), Little Greenbul ($n = 82$), Yellow-streaked Greenbul ($n = 178$), Placid Greenbul ($n = 138$), Striped-faced Greenbul ($n = 77$) and Yellow-throated Greenbul ($n = 90$).

We found notable differences in food selection of two of the six species of greenbuls between the dry and wet seasons. Two species fed more on fruit during the dry season. Shelley's Greenbul shifted from an omnivorous diet in the dry season to a predominant insectivorous diet in the wet season (Fishers' exact $p < 0.001$, **Table 2**), and Striped-faced Greenbul shifted from a predominantly frugivorous diet in the wet season to an omnivorous diet in the dry season (Fishers' exact $p < 0.001$, **Table 2**). Yellow-throated Greenbul was mainly frugivorous, but ate proportionally more fruits in the dry season. However, this was not statistically significant. In both seasons, Little Greenbul had a mixed insectivorous-frugivorous diet consisting of the same proportion of fruits and insects (Fishers' exact $p = 1$). The two *Phyllastrephus* species, Yellow-streaked Greenbul and Placid Greenbul, were both highly insectivorous; only twice was

TABLE 1 | Seasonal differences in occupancy for species participating in the 82 canopy mixed-species flock type.

Species names	Occupancy in wet season (%)	Occupancy in dry season (%)	Corrected *p*
Green Wood Hoopoe *(Phoeniculus purpureus)*	5	19	1
Green Barbet *(Stactolaema olivacea)*	40	29	1
Moustached Tinkerbird *(Pogoniulus leucomystax)*	30	21	1
Yellow-rumped Tinkerbird *(Pogoniulus bilineatus)*	28	10	1
Cardinal Woodpecker *(Dendropicos fuscescens)*	22	2	0.591
Olive Woodpecker *(Dendropicos griseocephalus)*	28	62	0.06
Dark Batis *(Batis crypta)*	8	21	1
Black-fronted Bushshrike *(Chlorophoneus nigrifrons)*	40	52	1
Grey Cuckooshrike *(Ceblepyris caesius)*	38	69	0.133
Common Square-tailed Drongo *(Dicrurus ludwigii)*	98	93	1
African Paradise Flycatcher *(Terpsiphone viridis)*	25	2	0.407
White-tailed Crested Flycatcher *(Elminia albonotata)*	18	12	1
Shelley's Greenbul *(Arizelocichla masukuensis)*	50	40	1
Yellow-throated Greenbul *(Arizelocichla chlorigula)*	2	24	0.542
Stripe-faced Greenbul *(Arizelocichla striifacies)*	42	17	0.337
Little Greenbul *(Eurillas virens)*	5	12	1
Yellow-streaked Greenbul *(Phyllastrephus flavostriatus)*	98	100	1
Yellow-throated Woodland Warbler ***(Phylloscopus ruficapilla)***	28	83	<0.001
White-winged Apalis *(Apalis chariessa)*	20	29	1
Black-headed Apalis *(Apalis melanocephala)*	98	83	1
Chapin's Apalis *(Apalis chapini)*	12	31	1
Brown-headed Apalis ***(Apalis alticola)***	12	93	<0.001
Southern Yellow White-eye *(Zosterops anderssoni)*	50	21	0.221
Waller's Starling *(Onychognathus walleri)*	0	14	NA
Uluguru Violet-backed Sunbird *(Anthreptes neglectus)*	12	5	1.0
Collared Sunbird *(Hedydipna collaris)*	10	14	1
Olive Sunbird *(Cyanomitra olivacea)*	10	10	1
Moreau's Sunbird *(Cinnyris moreaui)*	8	12	1
Dark-backed Weaver *(Ploceus bicolor)*	90	98	1

The occupancy corresponds to the seasonal percentage of mixed flocks in which we observed a focal species. The table only depicts species observed in at least five flocks. The p-values come from generalized linear models of the Bernoulli type testing for seasonal differences in species occupancy. We used Bonferroni correction on the p-values to minimize type-I error associated with multiple independent testing. Species showing significant differences in occupancy between the two seasons are highlighted in bold font. NA, not applicable, due to absence in flocks during the wet season.

the latter observed picking fruits (dry season), while Yellow-streaked was entirely insectivorous in both seasons, and an extremely active and numerous species in the study of canopy MSF's (**Table 2**). In contrast, the Placid Greenbul was an active and numerous member of the understory MSFs in the dry season. Thus, Fishers' exact test was not applicable for Yellow-streaked Greenbul and was highly non-significant for Placid Greenbul (Fishers' exact $p = 1$).

Mist-Netting

A total of 40,391 NMH was completed in the dry season versus 23,033 NHM in the wet season. Mist-netting was

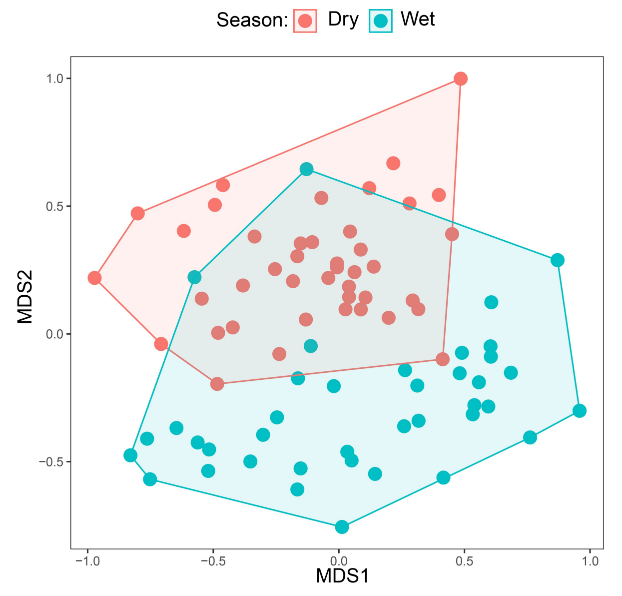

FIGURE 4 | NMDS plots comparing the species compositions of mixed flocks between the wet and dry season. Each dot corresponds to a mixed-species flock sampled in either the wet or dry season. Dots closer to each other have more similar species composition (measured by Bray–Curtis dissimilarity). The plot includes only canopy flocks as understory flocks were absent in the wet season.

TABLE 2 | Diet of six species of greenbuls in the dry and wet season in the Ndundulu study area including.

Greenbuls	**Number of observations per season**	
Shelley's Greenbul *(Arizelocichla masukuensis)*	**Dry**	**Wet**
Arthropods	63	73
Fruits	48	8
Little Greenbul *(Eurillas virens)*		
Arthropods	8	39
Fruits	5	30
Yellow-streaked Greenbul *(Phyllastrephus flavostriatus)*		
Arthropods	114	64
Fruits	0	0
Placidus Greenbul *(Phyllastrephus placidus)*		
Arthropods	103	33
Fruits	2	0
Striped-faced Greenbul *(Arizelocichla striifacies)*		
Arthropods	5	37
Fruits	20	15
Yellow-throated Greenbul *(Arizelocichla chlorigula)*		
Arthropods	3	5
Fruits	68	14
Sum	439	318

Total observations of all species and both categories are 757.

TABLE 3 | List of the 16 species caught in the mist-nets, showing the community turnover between the dry and wet season.

Species	**Dry season (*N* mistnetted)**	**Wet season (*N* mistnetted)**
Lemon Dove *(Columba larvata)*	0	14**
Moustached Tinkerbird *(Pogoniulus leucomystax)*	6	1
African Broadbill *(Smithornis capensis)*	0	20*
African Paradise Flycatcher *(Terpsiphone viridis)*	0	16*
White-tailed Crested Flycatcher *(Elminia albonotata)*	8	34
Shelley's Greenbul *(Arizelocichla masukuensis)*	90	53
Placid Greenbul *(Phyllastrephus placidus)*	38	19
Yellow-streaked Greenbul *(Phyllastrephus flavostriatus)*	0	8
Evergreen Forest Warbler *(Bradypterus lopezi)*	4	41
Cinnamon Bracken Warbler *(Bradypterus cinnamomeus)*	0	13
Bar-throated Apalis *(Apalis thoracica)*	12	33
Swynnerton's Robin *(Swynnertonia swynnertoni)*	8	2
White-starred Robin *(Pogonocichla stellata)*	3	111
Sharpe's Akalat *(Sheppardia sharpei)*	52	38
Olive Sunbird *(Cyanomitra olivacea)*	104	92
Oriole Finch *(Linurgus olivaceus)*	0	11
Species total	37	48
Total number of individuals caught (excluding recaptures)	674	956
Net meter hours (NMH)	23,033	40,391

$^{**}P < 0.01$, $^{*}p < 0.05$.

conducted at all mist-netting sites in both seasons (see the section "Materials and Methods"). The total number of individuals mist-netted was 1630, represented by 52 species: In the wet season 37 species of 675 individuals, and in the dry season: 48 species of 956 individuals (for accumulation curves, see **Figure 3**).

Four species were only seen during the dry season, while 15 other species were only observed during the wet season. The accumulation curve of species caught (**Figure 3**) indicates that mist-netting effectively catches the understorey species, apart from, e.g., gallinaceous birds. Certain species from higher strata were caught in small numbers, especially in the wet season, e.g., Striped-faced Greenbul, Yellow-throated Greenbul, Bar-throated Apalis, Oriole Finch, Yellow-streaked Greenbul, and White-tailed Crested Flycatcher.

We found a significant difference in species abundances between the two seasons: Chi-squared = 268.88, df = 38, *p*-value < 0.001. The statistical significance of the Chi-squared test was driven by 16 species having the largest change in relative abundance between the seasons. The overall result remains significant when any of these 16 species are included. If removing all 16 species, the overall Chi-squared test on the remaining 23 species is not significant (**Table 3**).

The number of singletons in the mist-nets was ten and doubletons three. Rarely captured species include: African Goshawk (*Accipiter tachiro*), Black-headed Apalis (*Apalis melanocephala*), Bar-tailed Trogon, Dapple-throat, Black-fronted Bushshrike, Klaas's Cuckoo, Olive Woodpecker, Collared Sunbird, Scaly-throated Honeyguide, African Dusky Flycatcher, Moustached Tinkerbird, Green Barbet, and Tambourine Dove.

Field Observation Data

The field observation data used for analysis (see **Table 4**) comprised a total of 70 species, with 65 species recorded in the wet season and 67 in the dry season. The results presented in **Table 4** show that Eastern Bronze-naped Pigeon *Columba delegorguei*, Barred Long-tailed Cuckoo *Cercococcyx montanus*, Bar-tailed Trogon, Green Barbet, African Broadbill *Smithornis capensis*, African Paradise Flycatcher *Terpsiphone viridis*, Stripe-faced Greenbul, Black-headed Apalis, Red-capped Forest Warbler, Orange Ground Thrush *Geokichla gurneyi,* and White-starred Robin *Pogonocichla stellata* were all more common during the wet than during the dry season. Of these, four species were almost absent from the montane forest during the dry season, namely Barred Long-tailed Cuckoo, African Broadbill, African Paradise Flycatcher, and White-starred Robin, while African Dusky Flycatcher was completely absent during the dry season.

DISCUSSION

Our data show that the montane forest bird community in the Ndundulu Mountains in the Udzungwas undergoes marked changes between the dry and wet seasons. This general result is clear in all the three independent studies: Some species move out of the montane forest in the dry season. Other species showed partial movements as they remained but with reduced numbers in the dry season and others again change diet or change the social behavior by participating or participating significantly more in mixed-species flocks. The seasonal turnover in community composition is particularly noticeable in the mixed-species flocks. As illustrated in the NMDS plot (**Figure 4**), only three canopy MSFs from the wet season were compositionally similar to flocks from the dry season. These were recorded between 15 and 30 November in the "short rains." In any case, our results support previous findings that the majority of the populations, including the vast majority of the Eastern Arc endemics – of which many are insectivorous species – do not undertake seasonal movements to lower altitudes (see **Table 4**, Annex 1, Burgess and Mlingwa, 2000). It has previously been shown that Udzungwa Mountains are home to a distinct montane bird community, with another community found at lower elevations (Romdal and Rahbek, 2009).

We are quite confident that key environmental drivers are responsible for the observed changes in species composition and behavior between seasons. The long dry season in the mountains lasts several months with minimal precipitation compared to a warm and humid wet season on average 5°C warmer in our study period 100-fold more rain and with most forest bird species breeding (Brown and Britton, 1980). This results in food scarcity in the dry season, especially for insectivorous species (see e.g., Brown and Britton, 1980; Williams and Middleton, 2008; see also Malizia, 2001). For instance, a study by Mulwa et al. (2012) found species richness and the total abundance of frugivores and insectivores concurred with the seasonal availability of their respective resources. In comparison, our data indicate that frugivorous-insectivore species are perhaps less influenced by seasonal variation, presumably because fruiting is more variable in time and space in the forest (see e.g., Adamescu et al., 2018), e.g., in different microhabitats such as light gaps, forest edge and between tree species. Thus trees fruiting varies not only between species and season but also within the same species and even the same tree from year to year (Wheelwright, 1986).

Comparable to frugivore-insectivorous, nectarivorous sunbirds may be similarly impacted by the variability of flowering (and thus nectar) and occurrence of invertebrates. They feed on both nectar from flowering plants/epiphytes and on insects and the more common species appear abundant in both seasons. There were indications that several of the rare sunbird species move more around in the non-breeding season and were recorded in the study area in the dry season only. However, the most common Olive Sunbird is known from other localities to undertake altitudinal movements (Cordeiro, 1994a), however, we cannot find it in our data for the Ndundulu Mountains.

We did not collect data on food abundance, either flowering nor, fruits, nor arthropods, and we have anecdotal information from the study area only: During the wet season, a rain of larvae droppings from above to the tarpaulin at camp could be heard. During the daytime, there were bursts of cicada sounds, butterflies were numerous and a different insect life compared to the dry season. Fruits and flowering seemed abundant in the dry season. These seasonal findings on food abundance seem to be supported by a study in forests in northern Kenya (Borghesio and Laiolo, 2004) and a study on tropical savannah birds in West Africa showed a positive response in breeding and body mass to resource availability brought about by seasonal rainfall (Cox et al., 2011), which concur with our findings in the Udzungwas (unpublished data).

Change in Behavior Between Seasons

Our results show that many, mainly insectivorous species, attend MSFs in the dry season. The distinct understorey MSF with the participation of birds from the forest understorey comprising up to 10 species and up to 26 individuals were active in the dry season only. Therefore, there seems to be a clear advantage of participating in MSFs during the dry season, e.g., related to the scarcity of food in that season. Likewise, the distinct canopy MSF comprised mainly canopy and higher mid-stratum species, and had more species and more individuals of each species in the dry season (e.g., 50 species in dry season versus 37 in the wet season). Furthermore, the mobility of MSFs was considerably higher in the dry season, and flocks moved faster through the forest compared to the wet season where the MSFs were virtually sedentary (TL unpublished data).

Some of the most abundant species in the bird community were the six greenbuls (see also Keith et al., 1992; Britton, 1980), which responded to the presumed change in food availability by a flexible behavior (see also Dinesen, 1997). Two of the six species studied changed diet and feeding behavior, see also Dinesen (1995) toward significantly more fruits in the dry season and there are indications that Yellow-throated Greenbul do the same as it almost entirely ate fruits in the dry season and occasionally fed on nectar and possibly leaves in this season compared to more arthropods in the wet season. An extremely rapid feeding behavior of Little Greenbul of sallying out swiftly hovering and catching airborne insects in the wet season seemed not to be practiced to the same extent in the dry season; however, more data are needed to support this. This seasonal shift in diet is in contrast to a study in a much drier forest environment in Kenya (Borghesio and Laiolo, 2004), where most species did not change their behavior between seasons. However, Borghesio and Laiolo (2004) nonetheless concluded that omnivores shifted to frugivory in the dry season and fed more on invertebrates in the wet season, as in our study.

The two insectivorous greenbul species Yellow-streaked Greenbul and Placid Greenbul were very characteristic and dominant members of each of the two distinct types of MSF's. Placid Greenbul ate fruits on a few occasions in the dry season, perhaps indicating the limitations of available arthropods in this season. The greenbul study, as well as the field and MSF data, support that these two insectivorous and gregarious greenbuls are more or less resident and abundant in both seasons (see also Dinesen, 1997).

TABLE 4 | Field observations in dry and wet season.

Species	Occupancy in wet season (%)	Occupancy in dry season (%)	*p*-Value	Corrected *p*-value
Udzungwa Forest Partridge *(Xenoperdix udzungwensis)*	26	44	0	1
Livingstone's Turaco *(Tauraco livingstonii)*	98	96	1	1
Klaas's Cuckoo *(Chrysococcyx klaas)*	2	0		NA
Barred Long-tailed Cuckoo *(Cercococcyx montanus)*	96	4		<0.001
African Olive Pigeon *(Columba arquatrix)*	43	29	0	1
Eastern Bronze-naped Pigeon *(Columba delegorguei)*	74	11		<0.001
Lemon Dove *(Columba larvata)*	32	5	0	0
Crowned Eagle *(Stephanoaetus coronatus)*	49	62	0	1
African Goshawk *(Accipiter tachiro)*	21	15	0	1
Mountain Buzzard *(Buteo oreophilus)*	43	47	1	1
African Wood Owl *(Strix woodfordii)*	0	4		NA
Bar-tailed Trogon *(Apaloderma vittatum)*	81	38		<0.001
Crowned Hornbill *(Lophoceros alboterminatus)*	55	44	0	1
Silvery-cheeked Hornbill *(Bycanistes brevis)*	94	96	1	1
Green Barbet *(Stactolaema olivacea)*	49	7		<0.001
Moustached Tinkerbird *(Pogoniulus leucomystax)*	94	82	0	1
Yellow-rumped Tinkerbird *(Pogoniulus bilineatus)*	11	7	1	1
Olive Woodpecker *(Dendropicos griseocephalus)*	11	16	0	1
African Broadbill *(Smithornis capensis)*	55	4		<0.001
Dark Batis *(Batis crypta)*	85	80	1	1
Black-fronted Bushshrike *(Chlorophoneus nigrifrons)*	45	24	0	2
Black-backed Puffback *(Dryoscopus cubla)*	4	4		NA
Fülleborn's Boubou *(Laniarius fuelleborni)*	89	69	0	1
Grey Cuckooshrike *(Ceblepyris caesius)*	28	35	0	1
Black Cuckooshrike *(Campephaga flava)*	0	5		NA
Common Square-tailed Drongo *(Dicrurus ludwigii)*	19	25	0	1
African Paradise Flycatcher *(Terpsiphone viridis)*	64	2		<0.001
White-tailed Crested Flycatcher *(Elminia albonotata)*	96	91	0	1
Shelley's Greenbul *(Arizelocichla masukuensis)*	89	85	1	1
Yellow-throated Greenbul *(Arizelocichla chlorigula)*	62	62	1	1
Stripe-faced Greenbul *(Arizelocichla striifacies)*	81	42		<0.001
Little Greenbul *(Eurillas virens)*	53	38	0	1
Placid Greenbul *(Phyllastrephus placidus)*	68	38	0	0
Yellow-streaked Greenbul *(Phyllastrephus flavostriatus)*	30	16	0	7
Dark-capped Bulbul *(Pycnonotus tricolor)*	21	18	1	1
Yellow-throated Woodland Warbler *(Phylloscopus ruficapilla)*	64	78	0	1
Evergreen Forest Warbler *(Bradypterus lopezi)*	98	91	0	1
Bar-throated Apalis *(Apalis thoracica)*	53	31	0	1
White-winged Apalis *(Apalis chariessa)*	0	4		NA
Black-headed Apalis *(Apalis melanocephala)*	83	15		<0.001
Chapin's Apalis *(Apalis chapini)*	68	40	0	0
Brown-headed Apalis *(Apalis alticola)*	6	18	0	1
Red-capped Forest Warbler *(Artisornis metopias)*	91	53		<0.001
Southern Yellow White-eye *(Zosterops anderssoni)*	64	35	0	0
Pale-breasted Illadopsis *(Illadopsis rufipennis)*	2	5		NA
Spot-throat *(Modulatrix stictigula)*	68	71	1	1
Dapple-throat *(Arcanator orostruthus)*	2	9	0	1
Waller's Starling *(Onychognathus walleri)*	96	73	0	0
Kenrick's Starling *(Poeoptera kenricki)*	21	13	0	1
Orange Ground Thrush *(Geokichla gurneyi)*	68	13		<0.001
Abyssinian Thrush *(Turdus abyssinicus)*	17	27	0	1
African Dusky Flycatcher *(Muscicapa adusta)*	34	0	1	1
White-chested Alethe *(Chamaetylas fuelleborni)*	6	5	1	1

(Continued)

TABLE 4 | (Continued)

Species	Occupancy in wet season (%)	Occupancy in dry season (%)	*p*-Value	Corrected *p*-value
Olive-flanked Ground Robin *(Cossypha anomala)*	40	9	0	0
Swynnerton's Robin *(Swynnertonia swynnertoni)*	19	25	0	1
White-starred Robin *(Pogonocichla stellata)*	96	4		<0.001
Sharpe's Akalat *(Sheppardia sharpei)*	53	35	0	1
Iringa Akalat *(Sheppardia lowei)*	19	9	0	1
Collared Sunbird *(Hedydipna collaris)*	15	16	1	1
Amani Sunbird *(Hedydipna pallidigaster)*	0	7		NA
Olive Sunbird *(Cyanomitra olivacea)*	64	67	1	1
Moreau's Sunbird *(Cinnyris moreaui)*	72	80	0	1
Rufous-winged Sunbird *(Cinnyris rufipennis)*	2	5		NA
Variable Sunbird *(Cinnyris venustus)*	0	22	1	1
Dark-backed Weaver *(Ploceus bicolor)*	32	27	1	1
Usambara Weaver *(Ploceus nicolli)*	17	0	1	1
Red-faced Crimsonwing *(Cryptospiza reichenovii)*	15	33	0	3
Oriole Finch *(Linurgus olivaceus)*	2	4		NA
Kipengere Seedeater *(Crithagra melanochroa)*	11	18	0	1

We used generalized linear models (GLM) with a log link function to test for seasonal differences in species-level occupancy (presence–absence observations per day). The occupancy analyses were conducted using a GLM based on a Bernoulli probability distribution and a logit link function. To ensure sufficient data for each analysis, we exclude species observed less than 5 days (n = 12). Due to the numerous independent tests, we apply Bonferroni correction on the p-values (i.e., multiplying the p-values by the number of conducted tests; n = 61). Species showing significant differences in occupancy between the two seasons are highlighted in bold font. NA, not applicable, due to absence in flocks during the wet season.

We speculate that the scarcity of arthropods in the dry season challenge insect-eating species and force them to either change diet, change social behavior, or move away. The vast majority of the MSFs comprised insectivorous species. These species may have a clear advantage of joining MSFs where many individuals moving through the forest increase the chance of flushing insects (Sridhar et al., 2009). The function of avian mixed-species flocks in Madagascar has been reported to change function between seasons from a function of increased foraging efficiency in the non-breeding period to a function of anti-predator defense in the breeding period (Hino, 2009). Moreover, some species might not have an advantage of joining MSFs during the breeding season, as arthropods are more abundant. A much less pronounced seasonal difference (dry versus wet season) is seen in Brazil (Maldonado-Coelho and Marini, 2000), which is probably partly due to the lower altitude of the forests, hence also a shorter altitudinal gradient compared to the Ndundulu Mountains.

There are indications from the mist-net results that some insectivorous understory species regarded as residents changed behavior between the seasons, for example, Placid Greenbul, Swynnerton's Robin *Swynnertonia swynnertoni*, and Sharpe's Akalat *Sheppardia sharpie.* These ground dwellers are expected to move more around in the dry season with no indications of moving out of the study area and were caught most frequently in the mist-nets in the dry season. Other insectivorous mid-stratum species were caught more frequently in the wet season, such as White-tailed Crested Flycatcher and Bar-throated Apalis. Both species we regard as residents in the study area, and we believe the reason is that these two mid-stratum species are generally feeding lower in the wet season, where many species place their nests close to the ground.

Lemon Dove *Columba larvata* forages on the ground on fruits. It shows a pronounced change in mist-net captures, with two in the dry versus 40 in the wet season. Fruits of some tree species are more abundant in the wet season (especially toward the last part of the season). Studies in coastal forests in South Africa have shown that the species is much more habitat-specific in the breeding season but abundance was overall the same (David et al., 2017; Smith et al., 2017). This may apply to our study area as well; however, there are also indications of seasonal movements in the Udzungwas (Stuart et al., 1987). The insectivorous Yellow-throated Woodland Warbler also changes social behavior with a significantly higher occupancy in the MSFs during the dry season. The species is abundant from the mid-stratum to the canopy, and there are hence no significant trends in the mist-netting data and neither in its general abundance in field observation data.

Seasonal Movements

It is interesting that Moreau (1935) stated in his paper on the Usamabara that "whether Forest or non-forest birds are considered, there appears to be practically no seasonal movement up and down the mountains." New evidence on movements have accumulated since (e.g., Burgess and Mlingwa, 2000 and many other references). Movement out of the montane forest is unambiguously shown by our mist-net and MSF data as well as field observations for five insectivorous species (6.3%) especially confined to the mid-stratum. These species appeared to leave the montane forest almost entirely, and they include: Barred long-tailed Cuckoo, African Broadbill, African Paradise Flycatcher, African Dusky Flycatcher, and White-starred Robin. White-starred Forest Robin is known to undertake downward movements on Kilimanjaro (Cordeiro, 1994b) and this species and African Dusky Flycatcher and are also known from South African forests and elsewhere to move to lower altitudes and African Paradise Flycatcher to be a breeding migrant (Craig and Hulley, 2019).

While our studies suggest that species leave the montane forest in the dry season, there are insufficient data/studies documenting where the birds move to. However, studies in the Uluguru Mountains have revealed an increase in the abundance of certain montane forest species in the foothills in the cold dry season (Werema et al., 2016) and in the foothills of the Udzungwa Mountains (Stuart et al., 1987). Observations in the lowlands during the dry season in Kimboza Forest Reserve and foothills of the Ukingu Mts (a.k.a. Nguru Mts) (LAH fieldwork, 2021) support the presence of many of the above-mentioned "montane" species. Burgess and Mlingwa (2000) were not able to tell whether occurrence in, e.g., coastal forests of possible seasonal migrants was based on a local resident population or the altitudinal migration from the montane areas of these species. Our perception of montane-lowland species is constantly challenged, e.g., with the observation of Swynnerton's Robin in the lowlands of East Usambara (Anderson et al., 1997) and findings of two montane species on Zanzibar island at ca. 35 m (LAH Fieldwork, 2021).

Our results also indicate that part of the population of certain other species may undertake seasonal movements between montane forest habitats and lowland forests. These species are for example: Bar-tailed Trogon, Stripe-faced Greenbul, Little Greenbul, Evergreen Forest Warbler *Bradypterus lopezi*, Cinnamon Bracken Warbler *Bradypterus cinnamomeus*, Black-headed Apalis, Red-capped Forest Warbler, and Orange Ground Thrush (see also Stuart et al., 1987).

The ground dwellers and insect-eating Cinnamon Bracken Warbler and Evergreen Forest Warbler were caught most frequently in the wet season, indicating a movement out of the area during the dry season. We find support for this from Lemaire (1989) who reported a regular downward shift of Cinnamon Bracken Warbler and Evergreen Forest Warbler in Malawi and the latter also by Cordeiro (1994a) for Tanzania.

The Brown-headed Apalis, a canopy species in the MSFs in the study area, was attending the MSFs significantly more during the dry season (see **Table 2**). In combination, our data indicate that there are considerably fewer individuals present in the forest during the wet season. Therefore, it seems that the majority of Brown-headed Apalis leave the forest presumably to breed somewhere else. No source we have been able to find seems to mention this species as anything but a "resident species" (e.g., Urban et al., 1997; Dowsett-Lemaire and Dowsett, 2006) (the latter reference as *A. cinerea*).

Other species may undertake movements within the montane forest and move down in altitude in the dry season. The field data does not indicate movements out of the study area of Yellow-throated Greenbul in the dry season. However, unpublished data by LAH show a seasonal change in relative abundance at certain altitudes within the altitudinal span of the study area. Within the complex of the Mountain Greenbul Lemaire (1989) and Dowsett-Lemaire and Dowsett (2006) did report "Eastern Mountain Greenbul *Andropadus nigriceps*" (now known as Black-browed Greenbul *Arizelocichla fusciceps*) to undertake a regular downward shift in Malawi, and Stuart et al. (1993) reported it to undertake altitudinal movements in the Ulugurus Mountains and small vertical seasonal migrations in the Usambaras.

A Conservation and Evolutionary Perspective

The continuous fragmentation of forests in East Africa, including the Udzungwa Mountains may, in connection with accelerating climate change have severe consequences for these tropical bird communities (see also Root et al., 2003; Williams and Middleton, 2008; Willig and Presley, 2016; Santillan et al., 2018). The only hypothetical escape for some species may be to move to higher elevations. There are few data to suggest that they do. Yet, the greatest loss of species from climate disruption may be for tropical montane species (Araújo and Rahbek, 2006; Peh, 2007; Forero-Medina et al., 2011). The seasonal dynamics of these bird communities demonstrate the need to take the full habitat requirements of all species into account in future conservation planning.

This study further sheds light on some of the larger patterns in relation to seasonal changes in East African montane forests exemplified by the montane bird community. The harshness of the long cold dry season can be seen as an evolutionary bottleneck for many species (see Williams and Middleton, 2008 for an Australian case). A marked seasonal variation in climate seems likely to favor species that can adapt to the different food supplies over the year. Other studies show that insectivorous species in MSFs are the most vulnerable to habitat fragmentation and/or destruction (Cordeiro et al., 2015). In a study in Kenya, Mulwa et al. (2012) concluded that bird communities strongly respond to seasonal fluctuations in resource availability, but responses differ between feeding guilds. While frugivorous birds seem to respond flexibly to seasonal fluctuations, for instance, by having the ability to track down fruit resources across habitat boundaries, insectivorous birds appear to be more susceptible to the increase in seasonal fluctuations in resource availability.

Several of the more common species are evolutionary generalists, capable of responding to different seasonal climates and feeding opportunities by a flexible behavior and ability to change diet. This behavior is exemplified by the greenbuls in this study (all abundant at certain altitudes), as some of these do both change their diet and feeding behavior and undertake partial movements. Another strategy is to "stay" in the montane forest and participate in MSFs, especially in the cold dry season, where we document a higher number of species and individuals to optimize feeding opportunities (see also Brown, 2014). In contrast, high precipitation in the high Andes seems to impose important constraints on birds (Santillan et al., 2018). Diet specialists may be more vulnerable as they cannot shift to another food source and therefore suffer to a greater deal when major habitat and climate changes occur (see also Dulle et al., 2016). There is, however, little doubt that the dry season in East Africa is challenging for many montane forest organisms, including insect-eating birds and their prey. A number of different evolutionary strategies have come into force to adapt to and survive this season, as demonstrated in this paper, and it may be a bottleneck reinforced in future by the increasing effects of climate change.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article is available as **Supplementary Material**.

AUTHOR CONTRIBUTIONS

LD, TL, and LH contributed to the conception and design of the study. JS and TR performed the statistical analysis. LD and LH wrote the first draft of the manuscript. TL wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.768020/full#supplementary-material

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The Limpopo–Mpumalanga–Eswatini Escarpment—Extra-Ordinary Endemic Plant Richness and Extinction Risk in a Summer Rainfall Montane Region of Southern Africa

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Climatic, edaphic, and topographic differences between mountains and surrounding lowlands result in mountains acting as terrestrial islands with high levels of endemic biota. Conservation of mountains is thus key to successful biodiversity conservation. The Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) in South Africa and the Kingdom of Eswatini is one of the largest components of southern Africa's Great Escarpment. Despite botanical collecting effort over 150 years, there has never been a holistic and comprehensive synthesis of plant endemics data for the LMEE. For the first time, we define the LMEE as an orographic entity, covering 53,594 km^2; it forms a contiguous highland area from the Pongola River in the south, north to the Woodbush area, and includes rugged western Eswatini. Using exhaustive literature mining, coupled with combined decades of fieldwork by the authors, and up-to-date taxonomic assessments of the 46 undescribed species, we provide the first robust list of plant endemics for the LMEE. The LMEE has 496 endemic plant taxa, comprising 10.7% of the provisional flora (4,657 taxa). This is more than double the endemic plant taxa in the Drakensberg Mountain Centre (DCM), and may be the richest concentration of montane endemics in southern Africa outside of the Core Greater Cape Floristic Region. Grassland hosts the largest number of endemics (74.2%), followed by Savanna (26.6%), then Forest (7.7%). Most endemics of conservation concern occur in Grassland (68.4%), in which one is Extinct and two are Extinct in the Wild. Evolutionary partitioning between Grassland, Savanna and Forest is suggested by low introgression of Biomes at family and genus level, and by a dominance of life-forms adapted to open habitats. High threat statuses for Grassland endemics can be attributed to the historical transformation of almost 20% of Grassland to forestry pre-1990, and ongoing degradation of primary Grassland.

With conservation area coverage only 11.1% of the LMEE, the exceptional richness of the endemic flora—combined with major conservation threats—suggest that the LMEE should become a major focus of conservation effort between South Africa and Eswatini as a matter of urgency.

Keywords: orographic, South Africa, Great Escarpment, conservation, grassland, savannah, forest, KwaZulu-Natal

INTRODUCTION

Because of the climatic, edaphic, and topographic differences between mountains and surrounding lowlands, mountains are often terrestrial islands with high levels of endemic biota (Merckx et al., 2015; Noroozi et al., 2018; Perrigo et al., 2019). Conservation of mountains is thus key to successful biodiversity conservation (Noroozi et al., 2018; Callisto et al., 2019). However, effective conservation planning is dependent on accurate biodiversity data (Eken et al., 2004; Underwood et al., 2018; Hoveka et al., 2020)—notably for range-restricted species that may be more vulnerable to extinction than widespread ones (Shrestha et al., 2019; Loiseau et al., 2020). For both ecological and historical biogeographical research streams, biogeographical studies require robust biodiversity inventories that can be used for analyses (König et al., 2019). Despite this, the biological diversity of many mountain systems globally—and especially those in southern Africa (Clark et al., 2011a; Hoveka et al., 2020)—remains inadequately documented. Lack of detailed and accurate biodiversity data has resulted from: (i) A general decline in baseline taxonomic collecting effort, in favor of using databases "as are"—especially for difficult-to-access mountains; (ii) a predominant focus on phytosociological methods, that by nature do not record fine-scale diversity; and (iii) digital herbarium records only becoming available recently (Hoveka et al., 2020).

There is a growing body of work in southern Africa that attempts to combine physical geography (in this case "escarpment mountains") and plant geography (in this case "endemism") (e.g., Clark et al., 2009, 2011b, 2014; Hahn, 2017, 2019; Carbutt, 2019), rather than matching endemism to "homogenous" vegetation units (e.g., the approach of Mucina and Rutherford, 2006). Determining the "mountain" (i.e., area) before the "endemic suite" is a well-established practice for determining mountain floras and patterns of endemism in mountains globally, despite it being seen with some skepticism by plant geographers. This approach provides the basis for mountain-specific statements relating to biodiversity ("mountains are rich in endemics"), and inclusion of mountains in key inter-governmental conventions (e.g., Agenda 21: Chapter 13, Intergovernmental Panel on Climate Change: High Mountain Areas, etc.; Hock et al., 2019). The challenge in southern Africa is that spatial delimitations informing endemism have been based on overall poor plant collecting coverage (Hoveka et al., 2020)—and a focus on macro-scale phytosociology—while fine-scale endemic-data remains under-appreciated. The exception is the celebrated Core of the Greater Cape Floristic region (hereafter referred to as the "Cape"; Born et al., 2007), which has seen an intense and sustained, c.350-year long focus on botanical exploration, discovery, taxonomic capacity, and an unrivaled appreciation for fine-scale endemism by a strong botanical community of practice (both professional and lay) (cf. Manning and Goldblatt, 2012; too many examples to mention here). Also, in the Cape region it is clear that physical geography (e.g., the Cape Fold Mountains) and plant geography (vegetation units associated to these mountains) overlay quite neatly (e.g., Mucina and Rutherford, 2006), thanks to the discrete linear sub-ranges surrounded (more or less) by pediplains on most sides (Linol and De Wit, 2016). Nevertheless, there would be value in teasing out the mountain contribution to endemism in the Cape Flora, to allow for equitable comparison, i.e., mountain endemism with mountain endemism—outside the Cape, regionally and globally.

"Simple" orographic delimitation is effectively used to define many of the world's great, discrete massifs (e.g., Körner et al., 2011). In South Africa, this works for the Cape Fold Mountains, but it is more complicated for the extra-Cape mountains due to their orogenesis—they are mostly passive post-Gondwana Escarpments, cuestas, or inselbergs (Partridge and Maud, 1987). Inselbergs could be considered "easy," but variation in size and elevation opens other discussions around inclusion, and inselberg swarms pose other problems. A more complex and nuanced method is thus required to define mountains or escarpments in southern Africa.

The mountainous north-eastern parts of South Africa and adjacent Kingdom of Eswatini (Eswatini)—formerly known as the "North-eastern Transvaal Drakensberg"—have been of long-standing fascination to botanists (Van Wyk and Smith, 2001). As a result, they have been relatively well-botanized over a period of some 150 years, and have a proven wealth of endemic species (e.g., Croizat, 1965; Deall and Backer, 1989; Matthews et al., 1993; Van Wyk and Smith, 2001; Dlamini and Dlamini, 2002). Various components of the area have been recognized for their endemic diversity and richness (e.g., Croizat, 1965)—largely along edaphic lines (e.g., Matthews et al., 1993)—leading to Van Wyk and Smith (2001) describing three Centers of Floristic Endemism: the Barberton, Sekhukhuneland, and Wolkberg Centers in this region. The Lydenburg Center was a later proposed addition by Emery et al. (2002), and these Grassland-focused centers have been incorporated into Mucina and Rutherford's (2006) "Northern Sourveld Endemics" concept. Despite this effort, there has never been a holistic and comprehensive synthesis of plant endemic data for this section of the southern African Great Escarpment (in South Africa and Eswatini)—which we term the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE). The aims of this paper are therefore to define the LMEE as a discrete orographic entity as part of the southern African Great Escarpment; provide the first robust list of plant endemics for the

LMEE; determine in which Biome most LMEE endemics occur; determine which life-forms dominate amongst the endemic taxa; and determine the level of conservation concern for the endemic flora of the LMEE.

MATERIALS AND METHODS

Defining the Limpopo–Mpumalanga–Eswatini Escarpment

The basic premise for the delimitation of the LMEE is based on geomorphology, i.e., an escarpment on which various biodiversity and ecological complexities occur. The starting point was the global dataset on mountainous areas, as defined and created by Körner et al. (2011). Because ruggedness (a change in elevation over a given distance) was the main determinant by Körner et al. (2011), we felt that it represented a rather crude indication of escarpments, as it focused on areas with a change in elevation rather than the identification of summits, hilltops or plateaus. Escarpments typically comprise relatively narrow areas where coastward planes meet interior plateaus, the rugged escarpment often merging seamlessly with the interior plateau (Ollier, 1982, 1991; Partridge and Maud, 1987; Moore and Blenkinsop, 2006). We therefore applied an approach where we combined "ruggedness" together with landscape position to more accurately delimit "escarpment mountains." A Digital Elevation Model (DEM) raster was used, with a resolution of 30 arc-seconds (∼1 km) and covered most of Africa south of the equator (U.S. Geological Survey's Center for Earth Resources Observation and Science., 1996). To determine ruggedness, we used ArcGIS Pro 1.3 and the geo-processing tool called *Focal Statistics* [using *Range* as statistics type with a Neighborhood search window of 8 × 8 cells, also as used in Körner et al. (2011)]. To calculate topographic position index (TPI) we used ArcMap 1.6 and the Land Facet Corridor Designer, v.1.2.884 add-in [from the Land Facet Corridor Designer toolbox of Weiss (2001) and Jenness et al. (2013)]. TPI reflects the difference in elevation between a focal cell and all cells in a defined search neighborhood; this approach is very useful for determining mountain summits, or higher-lying areas at a predetermined neighborhood search radius. The TPI was calculated using two search neighborhood distances to identify areas with the highest elevations (summit areas) within the surrounding landscape at distances of 200 km and 250 km. The TPI was then calculated at a 50 km neighborhood to determine regional/provincial scale summit and mountainous areas. The output GIS layers for each of the above GIS processes (2 × TPI and 1 × ruggedness) were rescaled to a common scale of 1–10 using the Rescale by Function tool in ArcGIS Pro. The values were summed using the *Weighted Sum* tool, effectively averaging the input from all three processes. This approach of rescaling and weighted sum is commonly used in habitat suitability analyses (Store and Kangas, 2001). Each layer was given the same weighting (33.3%). Output from the Weighted Sum tool was then classified using the upper standard deviation value limit to identify mountainous areas. The output was converted to a polygon, and cleaned up using a buffer distance of 750 m to eliminate small gaps/sinks. Fragments smaller than 35,000 ha were removed, and free-standing inselbergs were excluded (of which there are very few) resulting in a continuous study area boundary.

Listing the Endemics of the Limpopo–Mpumalanga–Eswatini Escarpment

It is important to be explicit about what one means by endemic before listing the endemic taxa of a geographical area. In this paper, "endemic species" means species confined to a particularly defined area based on current records and distributions (Anderson, 1994), while "near-endemic species" means species whose ranges are closely aligned with the same defined area as "endemic species" but not strictly confined to that area (Matthews et al., 1993; Carbutt and Edwards, 2006); near-endemics are much more subjective to define than endemics (Carbutt and Edwards, 2006). Endemism can be scaled geographically—"local (narrow) endemism," confined to a relatively small area (e.g., Table Mountain); "regional endemism," endemic to a larger area or region (e.g., southern Africa)—but also considered in terms of pre-defined vegetation units and biomes with areal delimitations, such as Grassland or Fynbos Biome endemics (e.g., Mucina and Rutherford, 2006).

A list of endemic species was compiled by extensive literature mining, building on the foundational works of *inter alia* (Burtt Davy, 1926; Croizat, 1965; Compton, 1976; Deall and Backer, 1989; Morrey et al., 1989; Matthews et al., 1993; Balkwill et al., 1997; Scott-Shaw, 1999; Van Wyk and Smith, 2001; Dlamini and Dlamini, 2002), merging data from these historical works, and updating the list with a combination of more recent taxonomic revisions, new species descriptions, and the expert knowledge of the authors (including not yet described endemic species). A total of 209 references (**Supplementary Material 1**) was used to build this list of endemic taxa. For many taxa, determining actual geographic distribution was difficult, and decisions as to endemicity were resolved using data from the South African National Biodiversity Institute [SANBI],[1] the African Plant Database,[2] herbarium records (BNRH, J), and unpublished data held by the authors (notably SS for Sekhukhuneland, KB for the Barberton area, and JB, BT, and ML for the Lydenburg area). This list was then refined by updating taxonomy through consultation with specialists, removing duplicates and synonyms, and citing the most relevant reference per species. Each endemic was assigned the most relevant publication citation, or—if not yet described—a herbarium specimen reference was provided. The final compilation was tabulated (**Supplementary Material 2**). For the sake of simplicity, we have not attempted to enumerate near-endemics in this study.

The ten families and genera with the largest number of taxa endemic to the LMEE were determined from **Supplementary Material 2**. The proportion of endemism

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[1] http://redlist.sanbi.org/

[2] https://www.ville-ge.ch/musinfo/bd/cjb/africa/recherche.php

in the LMEE flora was calculated using a provisional LMEE flora of 4,657 taxa (Timberlake and Clark, in preparation). We compared LMEE endemic floristic patterns with the Maloti-Drakensberg (Carbutt, 2019), being the only other large summer rainfall section of Escarpment with robust published data at this level. Endemism comparisons were made against other mesic southern African mountains for which endemism data are available, viz. the Cape Midlands Escarpment (Clark et al., in preparation), the Maloti-Drakensberg (Carbutt, 2019), the Manica Highlands (Timberlake and Clark, in preparation), Mount Mulanje (Strugnell, 2002), the Nyika Plateau (Burrows and Willis, 2005), and the Soutpansberg (Hahn, 2017). Comparative endemic richness (diversity by region) was plotted and graphed using log-transformed data.

Determining Life-Forms

Each endemic taxon was assigned a life-form based on a modified version of the Raunkiaer system (Raunkiaer, 1934). An additional category under Chamaephytes was added: geoxyles (geoxylic growth forms of woody subshrubs characterized by massive lignotubers, or underground woody axes, from which emerge aerial shoots which may be ephemeral), given the large number of species in this life-form category in the LMEE (and elsewhere in Africa) (Olivier et al., 2014) and their misfit in the existing Raunkiaer system. Proportions of life-forms in the endemic flora were calculated.

Determining Distribution of Endemics in Biomes

The Biomes present [Grassland, Savanna, and Forest; as defined by Mucina and Rutherford (2006)] and their extent in the LMEE were identified, calculated and mapped using the latest version of the South African Vegetation map. In the LMEE, "Savanna" includes lightly wooded areas on otherwise grassy mountain slopes as well as the conventional "vegetation with a grass-dominated herbaceous layer and low to tall trees" (Mucina and Rutherford, 2006). The endemic taxa were assigned to each of the three Biomes; to achieve this we used a combination of descriptions in the endemic literature (**Supplementary Material 1**), together with personal field experience of the authors, and from the labels of herbarium vouchers (at BNRH and J, and using JSTOR Global Plants). Proportional representation per Biome was determined accordingly, at family, generic, and (infra-)specific levels.

Determining Level of Conservation Concern

The Red Data category (International Union for Conservation of Nature [IUCN], 2021) of each endemic taxon was collated from the SANBI Threatened Species Programme for taxa occurring in South Africa, and from Dlamini and Dlamini (2002) for taxa only occurring in Eswatini. From this, the proportion of endemic taxa per Red Data category was determined. Biome and life-form (see below) partitioning among the categories in the threatened bundle (E, EW, CR, EN, VU, LRnt) were specifically determined.

As land transformation is typically the largest threat to the conservation of narrow endemics (Newbold et al., 2018), land-cover change (1990–2020) was determined for the LMEE (for South Africa only, as there is no data for Eswatini) to determine trends over these 30 years; the datasets were sourced from Thompson (2016, 2021) for the years 1990 and 2020 and analyzed in ArcGIS Pro. The 1990 and 2020 land-cover classes were first standardized into fewer common classes, before determining the size of each class and then calculating the rate of change. A current protected area (PA) map (formal PAs in addition to conservation areas (CA), i.e., those with conservation agreements but not formally gazetted as PAs) and proportion of the LMEE under conservation management was generated using the South Africa Protected Areas Database (SAPAD; Department of Forestry, Fisheries and the Environment [DFFE], 2021a) and the South Africa Conservation Areas Database (SACAD; Department of Forestry, Fisheries and the Environment [DFFE], 2021b), as an indicator of conservation success (or otherwise) in the LMEE. In addition, as climate change is a major risk to areas with high local endemism (Manes et al., 2021), anticipated long-term changes to rainfall patterns were determined using a linear trend in mean annual precipitation values over 39 years (1981–2020), and expressed as a rate of change for each pixel in the multidimensional raster [using CHIRPS data from Funk et al. (2015)]. The analysis was run using the Generate Trend Raster geoprocessing tool in ArcGIS Pro. Additionally, from literature and authors' knowledge of the LMEE, general conservation threats to the endemic flora were identified.

RESULTS

Definition of the Limpopo–Mpumalanga–Eswatini Escarpment

Covering 53,594 km^2, the LMEE comprises that component of the eastern Great Escarpment in South Africa and Eswatini occurring between the Pongola River in the south, Woodbush in the north, the Highveld in the west, and the Lowveld in the east (23°31′ ′23″ ″S–27°49′ ′S and 29°50′ ′E–31°37′ ′E; **Figure 1** and **Supplementary Plate 1**). The rugged, northern components were much easier to define than the southern components, due to the seamless merger in the south-west and south with the interior Highveld Plateau, and less of a distinct Escarpment edge in Eswatini in the south-east. More specifically, the study area is a complex region with a very irregular boundary that straddles the northern-most edge of KwaZulu-Natal (KZN) province in South Africa, western Eswatini, and almost all of the mountainous areas of Mpumalanga province in South Africa, and a good proportion of the mountainous areas in Limpopo province (those occurring outside the study area in this province being the Soutpansberg, Waterberg, and western Strydpoortberge). Just over half (53%) of the LMEE occurs in Mpumalanga province, followed by Limpopo province (22%), and KZN and Eswatini with roughly equal amounts (13 and 12%). Significantly, the LMEE comprises 37% of the surface area

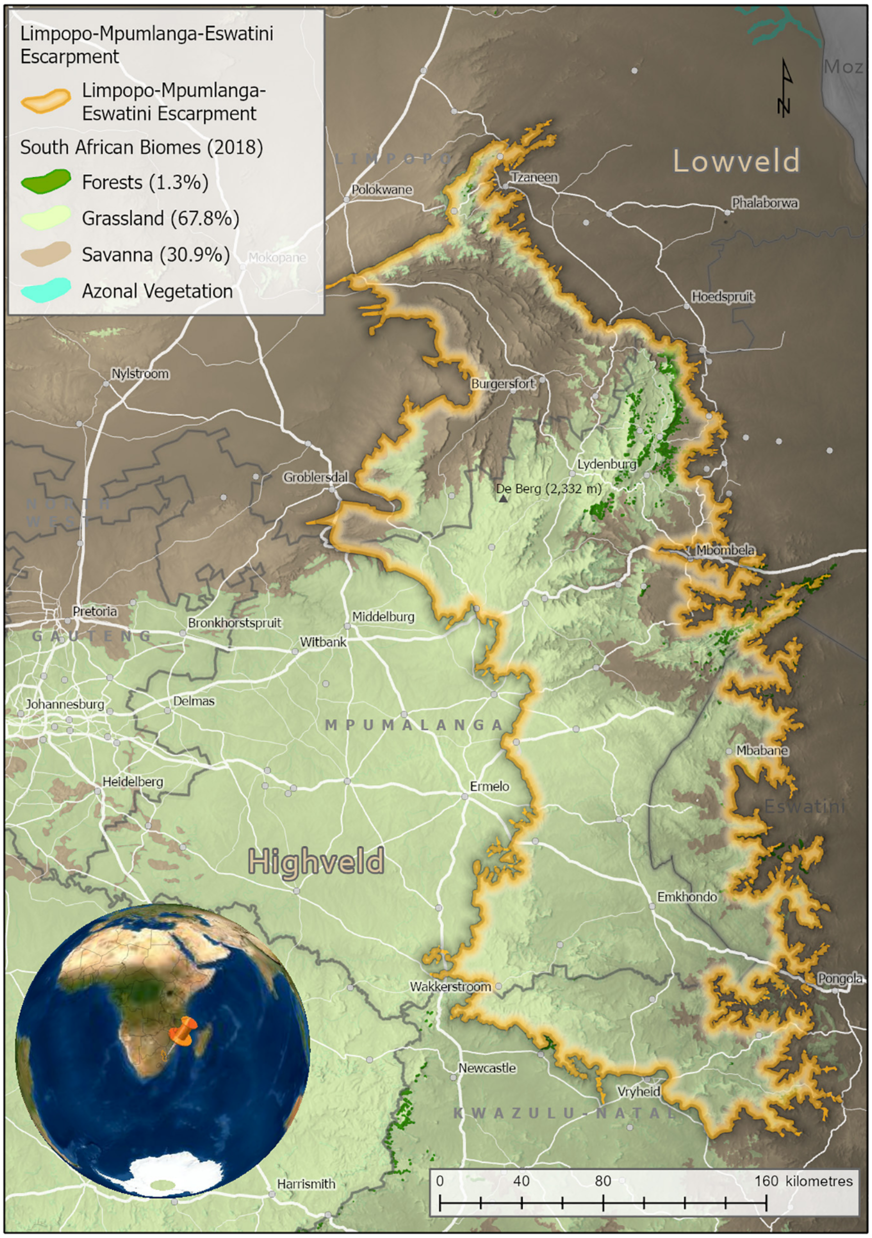

FIGURE 1 | The Limpopo–Mpumalanga–Eswatini Escarpment (LMEE), South Africa, and Eswatini, showing key cadastral features and Biomes (percentages are of the total LMEE area). Biomes derived from the Vegetation of South Africa, Lesotho, and Swaziland (Mucina and Rutherford, 2006). Elevation values are displayed in dark (lower elevations) to light shading (higher elevations).

in both Mpumalanga province and Eswatini—indicating high significance for provincial and national policy for each (e.g., Emery et al., 2002)—but only 10% of the surface area of Limpopo province, and 7% of KZN.

In the south, the LMEE is primarily of low topographic heterogeneity, comprising large, flat higher elevation areas stepping down relatively gently to the Eswatini Lowveld in the east and the KZN Middle-veld in the south. In the south-west it merges seamlessly with the uniform eastern Highveld plateau. The LMEE becomes progressively more topographically variable further north: the Barberton Mountainlands, lying perpendicular to the main Escarpment, run into northern Eswatini, while north of here the dramatic eastern ramparts of the "Transvaal Drakensberg" take shape from Kaapsehoop, and run north to the Wolkberg; this spectacular Escarpment is made more complex by numerous deep incisions from fluvial erosion, the most dramatic being the Olifants River valley and the Blyde River Canyon. North of the Wolkberg the Escarpment tapers into the lower-elevation, less rugged Haenertsburg–Woodbush–Modjadji area. In the far north-east, the LMEE ends at the

"Nkumpi Interval" in the Strydpoortberge—separated from the further western mountains by this major fluvial incision. Compared to the south-west, the central-west and north-west are much more complex, adding major topographic interest to the study area in the form of the rugged Sekhukuneland area (part of the eastern limb of the Bushveld Igneous Basin). The highest point in the LMEE is "De Berg" (2,332 m) on the Steenkampsberg (**Figure 1**).

The biophysical environment of the LMEE has been well documented by many authors [too many to mention here, but see Deall and Backer (1989), Matthews et al. (1993), Van Wyk and Smith (2001)]. In summary, the LMEE is geologically extremely diverse, hosting some of the oldest geological formations currently recognized on earth (viz. the Barberton Greenstone Belt), and an array of formations that include basement granites, serpentinites, ultramafics, carboniferous rocks, various metamorphics, and sedimentary sequences (De Wit et al., 2011). Climatically, the LMEE is temperate to sub-tropical, with strong temperature and moisture gradients across the Escarpment from the Lowveld (hot and dry) in the east to the Highveld (cool and moist) in the south-west, and Bushveld (warm and semi-dry) in the north-west. The LMEE supports various bioregions and vegetation units associated with the Grassland, Savanna and Forest Biomes (Mucina and Rutherford, 2006).

Land-use in the LMEE is complex (**Figure 2**), comprising a mix of commercial forestry (12.6%), conservation land (e.g., Blyde River Canyon, Buffelskloof Private, and Lekgalameetse Nature Reserves; Wolkberg Wilderness Area; Blyde to Kruger Biosphere Reserve; and numerous Natural Heritage Sites—a total of 11.1% of the area), areas under customary law, small to medium-sized urban areas, rangeland, commercial and subsistence agriculture, mining, and tourism (centered on natural scenery—the study area is famous for its vistas, waterfalls, hiking trails, biodiversity, and historical and cultural sites). The LMEE is also the main provider of fresh water to the Lowveld, notably for large-scale irrigation of tropical fruit production and the rivers of the Kruger National Park. The area has a rich cultural, literary and historical heritage, being notable for several gold rushes in the later 1800s; the fabled "missing Kruger millions" of President Paul Kruger (last president of the Zuid-Afrikaanse Republiek, until 1900); the legendary Modjadji Rain Queen; and Anglo-Boer War battles. The rich history of the area has been immortalized in literary works such as *Jock of the Bushveld* (FitzPatrick, 1907), *Lost Trails of the Transvaal* (Bulpin, 1956), and *Valley of the Mists* (Klein, 1972), and the lore and cultures of the Ndebele, Pedi, Shangane and Swazi people groups.

List of Endemics of the Limpopo–Mpumalanga–Eswatini Escarpment

The LMEE has 496 endemic taxa, representing 52 families and 158 genera (**Table 1** and **Supplementary Material 2**). This list includes two Pteridophytes, nine Gymnosperms (all Zamiaceae), and 485 Angiosperms; the latter are dominated by Dicotyledons (321, compared to 164 Monocotyledons). As a comparison, the DMC has six endemic Pteridophytes (Crouch et al., 2011), but no endemic Gymnosperms. There are no endemic families or genera in the LMEE—compared to no endemic families and two endemic genera in the DMC (Carbutt, 2019). However, the monotypic genus *Prototulbaghia* (currently sunk under *Tulbaghia*, Alliaceae; Stafford et al., 2016) may be a good genus (Vosa et al., 2011), and if resurrected would be an endemic genus in the LMEE. The LMEE is the richest montane area in southern Africa for Zamiaceae, with nine endemic taxa.

Asteraceae is the family with the most endemic taxa (57), followed by Lamiaceae (46) and Apocynaceae (42) (**Figure 3A**); this is contrary to the DMC, which has Scrophulariaceae and Iridaceae as second and third families (Carbutt, 2019). In the LMEE, 30 genera have ≥ 5 endemic taxa, and the three genera with the most endemic taxa are *Aloe*, *Helichrysum*, and *Ledebouria*; this compares to *Helichrysum*, *Senecio*, and *Delosperma* in the DMC (Carbutt, 2019). There are several tied contenders for the top ten endemic-rich genera, for example three share eighth place (*Asparagus*, *Disa*, *Encepharlatos*), four ninth place (*Euphorbia*, *Ocimum*, *Searsia*, *Selago*), while two tenth place (*Protea*, *Senecio*) (**Figure 3B**).

Considering the estimated 56,451 vascular plant species known for the Afrotropical flora (Raven et al., 2020), the LMEE flora of 4,657 taxa contributes 8.25% to this continental diversity and comprises 23% of the South African flora (Klopper, 2021). Endemism comprises 10.7% of the provisional LMEE flora (**Table 2**). This may be the richest concentration of montane endemics in southern Africa outside of the Core of the Greater Cape Floristic Region (Manning and Goldblatt, 2012)—being more than double the number of endemics in the DMC, and having the highest endemic richness of any other summer rainfall mountain system in southern Africa for which we have data. While the slightly larger area size of the LMEE compared to the DMC and the Cape Midlands Escarpment (CME) may contribute to its richer endemism, area—as indicated by Clark et al. (2014)—appears overall to be a weak predictor of endemic richness in southern African mountains (**Supplementary Material 3**). Also, our refined LMEE endemics tally (compared with Clark et al.'s 2014 estimate) places the LMEE quite superior to the DMC.

Distribution of Endemics Among Biomes

Grassland is by far the dominant Biome in the LMEE (74.2%), covering large parts of the center and south, with a thin wedge extending along the Escarpment rim to the north (**Figure 1**). Although Savanna dominates in the north and central east, it is only a third (26.6%) of the total LMEE. Forest is by far the smallest Biome (7.7%), being confined to comparatively small patches as Mucina and Rutherford's (2006) Northern Afrotemperate Forest (KZN–Mpumalanga border), Scarp Forest (mostly in Eswatini), and Northern Mistbelt Forest (remainder of the LMEE) along the prevailing/south-facing parts of the LMEE, and in riparian environments; it is worth noting that some of the largest patches of evergreen forest in South Africa outside of the southern Cape occur in the LMEE, notably in the Mariepskop and Wolkberg areas (cf. Van Wyk and Smith, 2001). Given that Grassland is the dominant Biome, it is perhaps not surprising then that this is the overwhelmingly dominant habitat for

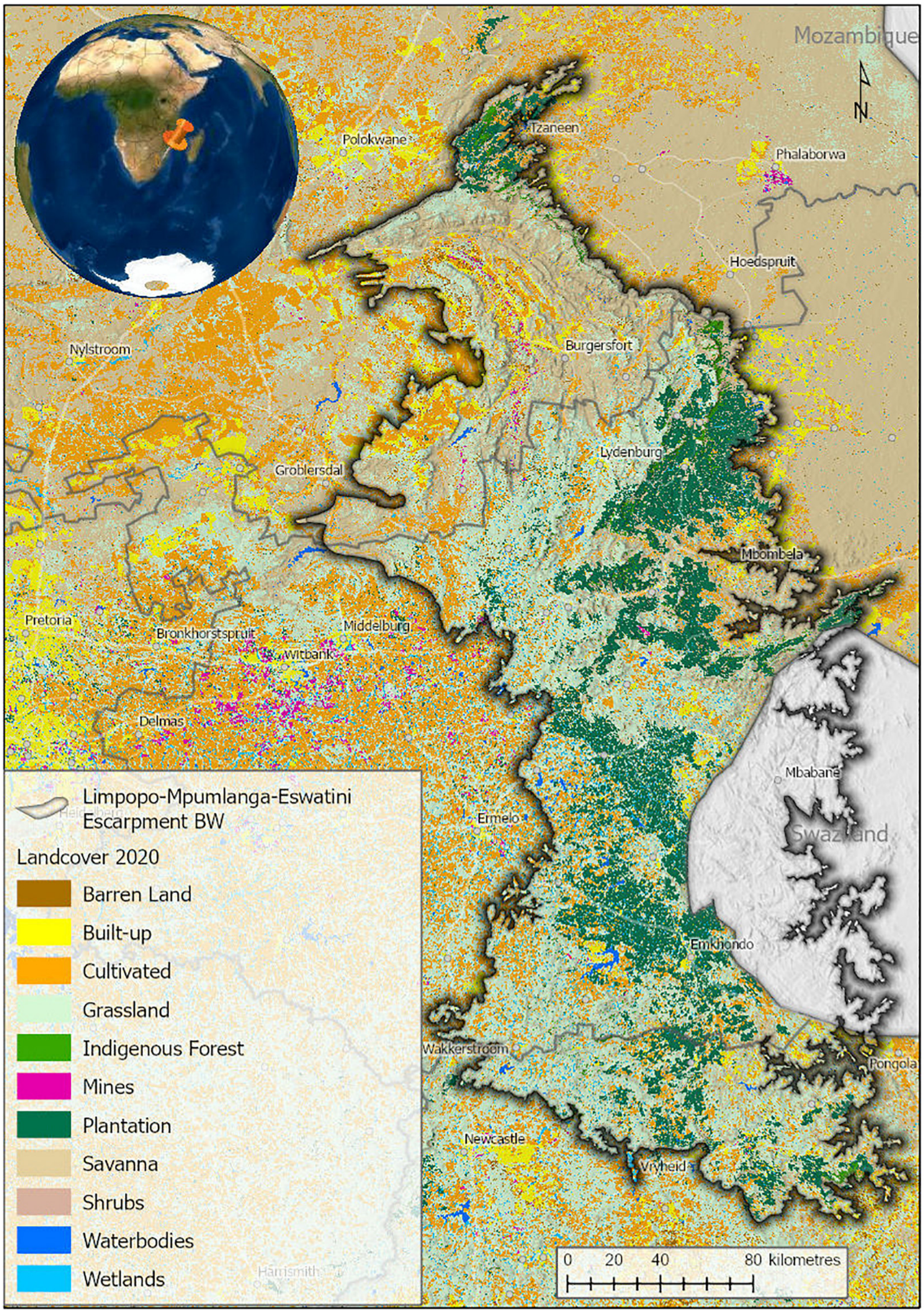

FIGURE 2 | Land-cover in the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) in 2020. Land-cover generated from Sentinel 2 satellite imagery for South Africa (Geoterraimage, 2021).

LMEE endemics, hosting almost three-quarters of the endemics, while Savanna hosts a quarter, and Forest less than ten percent (**Figure 4**). Different families dominate the lists of largest families in each Biome, with only Asteraceae and Lamiaceae being a top five family in all three Biomes (**Supplementary Material 4A**). Similarly, the top five genera with endemics per Biome are well partitioned, although *Aloe* occurs in the top five of each Biome and *Helichrysum* occurs in two (**Supplementary Material 4B**).

TABLE 1 | Distribution of endemics per major plant group among Biomes in the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE).

Plant group		LMEE	Forest	Savanna	Grassland
Pteridophytes	No.	2	1	1	0
	%	0.4	0.2	0.2	0
Gymnosperms	No.	9	1	1	7
	%	1.8	0.2	0.2	1.4
Angiosperms	No.	486	36	130	361
	%	98	7.3	26.2	72.8
Dicotyledons	No.	321	27	91	238
	%	64.7	5.4	18.3	48
Monocotyledon	No.	165	9	39	123
	%	33.3	1.8	7.9	24.8
Total	No.		38	132	368
	%		7.7	26.6	74.2

% indicates the proportional contribution made by each plant group to endemism levels of the LMEE or Biome.

Life-Forms

Of the 16 life-form categories used, four life-forms together represent 62.5% of all endemics, each of the four having more than 10% of the endemic taxa (**Figure 5** and **Supplementary Plates 2**, **3**). Caespitose Hemicryptophytes are the most numerous life-form, characterized by genera such as *Helichrysum*, which is a typical Grassland genus in the LMEE (**Supplementary Material 4B**). The second most numerous are bulbous Cryptophytes (geophytes), characterized by the rich petaloid monocotyledonous flora in the LMEE—represented in the top ten genera by *Ledebouria*, *Gladiolus*, and *Disa*; in the LMEE, these are typically Grassland and Savanna taxa (**Supplementary Material 4B**). Petaloid monocotyledons are also well represented in the LMEE by Iridaceae, Asparagaceae, and Orchidaceae—being in the overall top ten families (**Figure 3A**), also with a dominance of Grassland taxa, but with a strong Savanna component (**Supplementary Material 4B**). The third-largest group—woody Phanerophytes—are represented in the top 10 genera by *Syncolostemon*, *Encephalartos*, *Searsia*, and *Protea*, and again, many of these constitute Grassland rather than Savanna species (**Supplementary Material 4B**). Succulent Chamaephytes—the fourth-largest life-form—is best represented by *Aloe*, another primarily Grassland genus in the LMEE (**Supplementary Material 4B**).

Level of Conservation Concern

We found that a total of 74.8% of the LMEE endemics have been assessed for conservation status, the remaining species being Data Deficient or Not Assessed (combined 25.2%). Currently, 191 endemic plant taxa are under some form of risk in the LMEE. The single largest group—almost a third—is Least Concern (**Figure 6A**). However, the combined total of those with a threat status from Near-Threatened to Extinct is more than a third of the endemics (38.5%), with almost a quarter in this threat bundle being Vulnerable (**Figure 6A**). It may also be that the number of taxa in the threat bundle may increase further, once the Data Deficient and Not Assessed taxa are assessed (particularly the novel taxa still to be described, many of which are narrow endemics). The Rare category—usually local endemics without immediate threats—while below 10% is still very significant, and these species could easily join categories of higher concern in time through loss of habitat. *Pachycarpus stelliceps* (Apocynaceae; endemic to Eswatini) is a Grassland taxon that has not been re-discovered in the type locality area of Mbabane; its identity is doubtful and it might remain a taxonomic mystery, but it is currently recognized as the only Extinct taxon in the LMEE. The genus (and family) with the most threatened taxa in the LMEE is *Encephalartos* (Zamiaceae): of the nine endemics, two (*Encephalartos brevifoliolatus*, *E. nubimontanus*) are Extinct in the Wild, five are Critically Endangered, and two are Vulnerable.

Proportion of conservation statuses per Biome again mirrors the dominance of Grassland by a very large margin (68.4%), followed by Savanna (24.5%), then Forest (7.1%). Taking just the threat bundle, Grassland has the most "at-risk" taxa in any of these categories (**Figure 6B**). While this may be simply a factor of area (a larger Grassland area will naturally host more endemics at risk than the other two Biomes), historically Grassland has seen the largest transformation in the LMEE. Much of this transformation can be attributed to afforestation for timber (Armstrong et al., 1998; **Figure 2**), which in 2020 accounted for 18.9% of the LMEE area (**Supplementary Material 5**). Although there was only an increase of 1.3% in total afforested area between 1990 and 2020 (**Supplementary Material 5**), the historical transformation of Grassland before 1990 represents a probably irreversible loss of a substantial amount of this Biome—with detrimental impacts on endemic flora and fauna (e.g., the Extinct *Tetradactylus eastwoodae*, Eastwood's Long-tailed Seps lizard, in the heavily afforested Woodbush area; Bates et al., 2014). Coupled with other forms of transformation, an additional 3% of the LMEE that was previously Grassland was lost between 1990 and 2020 (**Supplementary Material 5**). Savanna experienced a loss of 3.4% of the area of the LMEE between 1990 and 2020 (likely due to increased mining and agricultural activities in that time—the latter being the biggest change in the LMEE over 30 years). It is worth noting that (indigenous) Forest expanded by 0.2% of the area of the LMEE in this time.

Only 10.4% of the LMEE is under formal conservation, with an additional 0.7% under stewardship conservation agreements (there is substantial overlap between the two categories in the Kruger-to-Canyons Biosphere Reserve). The distribution of PAs/conservation areas in the LMEE is scattered (**Figure 7**).

The LMEE has areas of marginal increase in precipitation over 39 years, and areas of marginal decrease (**Figure 8**). These changes are not confined to any one Biome, with Grassland in the southern areas and on the Escarpment edge experiencing a decrease, while Grassland in the central-west an increase. Similarly, embedded Forest in these areas would experience the same effect as the surrounding Grasslands (positive or negative). Savanna, in contrast, seems to have experienced primarily a marginal increase in precipitation. As climate change may increase the risk of extinction for LMEE endemics, it is suggested that Grassland endemics localized to the south and extreme east of the LMEE are most at risk of climate change extinction, assuming that

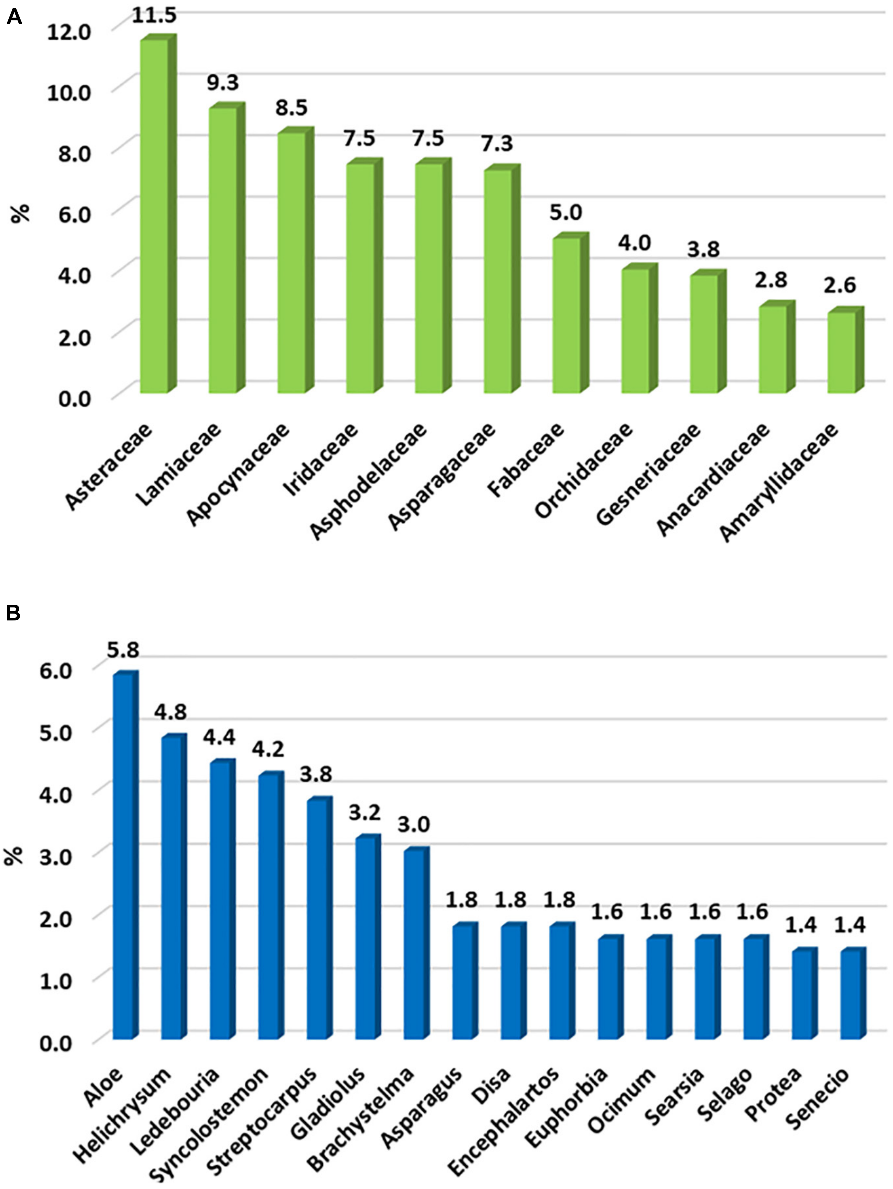

FIGURE 3 | The top ten **(A)** families and **(B)** genera hosting the most endemic plant taxa in the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE); we include shared results, resulting in 11 families and 16 genera holding the top ten positions.

LMEE endemism is largely dependent on consistent summer moisture availability.

DISCUSSION

Definition of the Limpopo–Mpumalanga–Eswatini Escarpment

This work in the LMEE shows that it is possible to pragmatically delineate a section of Escarpment (mostly) objectively as a discrete orographic entity, and to define an endemic suite of species associated to it. The most similar example on the scale of our LMEE is Carbutt's (2019) overlay of the Maloti-Drakensberg (i.e., discrete massif) as equal to his DMC—eliminating the original Drakensberg Alpine Center with its arbitrary 1,800 m lower contour (Van Wyk and Smith, 2001), but including a new alpine sub-center based on an isotherm and associated approximate elevation (2,800 m); there is thus a blend in the DMC between a physical (geomorphological) lower boundary (the base of the Maloti-Drakensberg), and an internal division based on an ecological indicator (an isotherm representing the lower bounds of the alpine zone). Other examples on smaller spatial scales are several of Van Wyk and Smith's (2001) Centers: the Kamiesberg Center = Kamiesberg Escarpment

TABLE 2 | Selected comparison of Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) floral endemism with that of other mesic southern African mountains, arranged in descending order of areal extent.

Mountain System	Number of Endemics			Total Endemics	Flora	Endemism (%)	Area (km²)
	Pteridophytes	Gymnosperms	Angiosperms				
LMEE	2 (This paper)	9 (This paper)	485 (This paper)	496	4,657 (Timberlake and Clark, in preparation)	10.7	53,594
Drakensberg Mountain Center	6 (Crouch et al., 2011)	0	229 (Carbutt, 2019)	235	?	c.9 (Carbutt, 2019)	36,478 (Carbutt, 2019)
Cape Midlands Escarpment	0 (Crouch et al., 2011)	1 (Clark et al., in preparation)	94 (Clark et al., in preparation)	95	c.2,000	4.75	31,500
Manica Highlands	0 (Crouch et al., 2011)	1 (Goode, 1989)	221 (Timberlake and Clark, in preparation)	222	c.2,500	9	14,621 (Timberlake and Clark, in preparation)
Soutpansberg *sensu lato*	0 (Crouch et al., 2011)	0 (Hahn, 2017)	44 (Hahn, 2017)	44	2,454 (Hahn, 2019)	1.79	6,700 (Hahn, 2017)
Nyika Plateau	0 (Burrows and Willis, 2005)	0 (Burrows and Willis, 2005)	33 (Burrows and Willis, 2005)	33	1,817 (Burrows and Willis, 2005)	1.82	5,451 (Google Earth)
Mount Mulanje	0 (Strugnell, 2002)	1 (Strugnell, 2002)	68 (Strugnell, 2002)	69	1,303 (Strugnell, 2002)	5.3 (Strugnell, 2002)	640 (Strugnell, 2002)

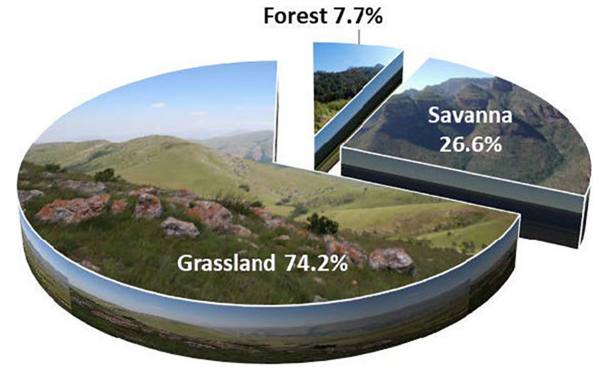

FIGURE 4 | Partitioning of endemic plant taxa per Biome (Grassland, Savanna, and Forest) in the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE).

(Namaqualand); the Hantam–Roggeveld Center = south-western Karoo Escarpment (later revised by Clark et al. (2011b) as both a physically and ecologically defined area); the Great Dyke Center (Zimbabwe) = a linear, igneous mega-intrusion, and the Chimanimani–Nyanga Center = (more or less) the Manica Highlands (Zimbabwe–Mozambique; currently being revised by Timberlake and Clark, in preparation). Similarly, Hahn; Hahn's (2017; 2019) Soutpansberg Center = the Soutpansberg *sensu lato* (Limpopo Province); Van Staden et al.'s (2020) Griqualand West Center = three mountain ranges of differing geology; Clark et al.'s (2009) Sneeuberg Center = Sneeuberg mountains (Eastern Cape); and Clark et al.'s (2014) Great Winterberg–Amatholes (GWA; Eastern Cape), conservatively held back from being a potential Center, perhaps better as part of a revised larger Center including the Sneeuberg, Stormberg and GWA (the "Cape Midlands Center"—also an orographically defined concept; Clark et al., in preparation). What is significant in all of these is that the orographic definition precedes or is (nearly) equal to the endemic circumscription. From first principles of biogeography this is reasonable, as endemism is dependent on a pre-defined area (Anderson, 1994)—however, it should not be confused with the process of defining a Center of Endemism (Linder, 2001), which requires a different departure point based on an analysis of the spatial distribution of taxa (e.g., Van Wyk and Smith, 2001; Mucina and Rutherford, 2006). It is not always clear—however—in southern Africa's Centers of Endemism as to which process has been followed, and the Center of Endemism concept is likely overdue for a complete reconsideration, redefinition, and re-analyses at a regional scale based on first principles. In this regard, we are cautious here and do not claim the LMEE as a Center of Endemism, or a replacement Center for the existing three Centeres associated to the LMEE. Nevertheless, it is generally agreed in biogeography that there is a high congruency between "mountains" and "endemism" (Noroozi et al., 2018), and mountains in southern Africa (both Cape and extra-Cape) are clearly important focal areas of endemism, and thus for biological conservation (Van Wyk and Smith, 2001; Mucina and Rutherford, 2006, etc.).

List of Endemics of the Limpopo–Mpumalanga–Eswatini Escarpment

The extra-Cape mountains in southern Africa have not benefited from the same enthusiasm for ongoing taxonomic discovery, inventory, and focus on narrow endemism at the same pace

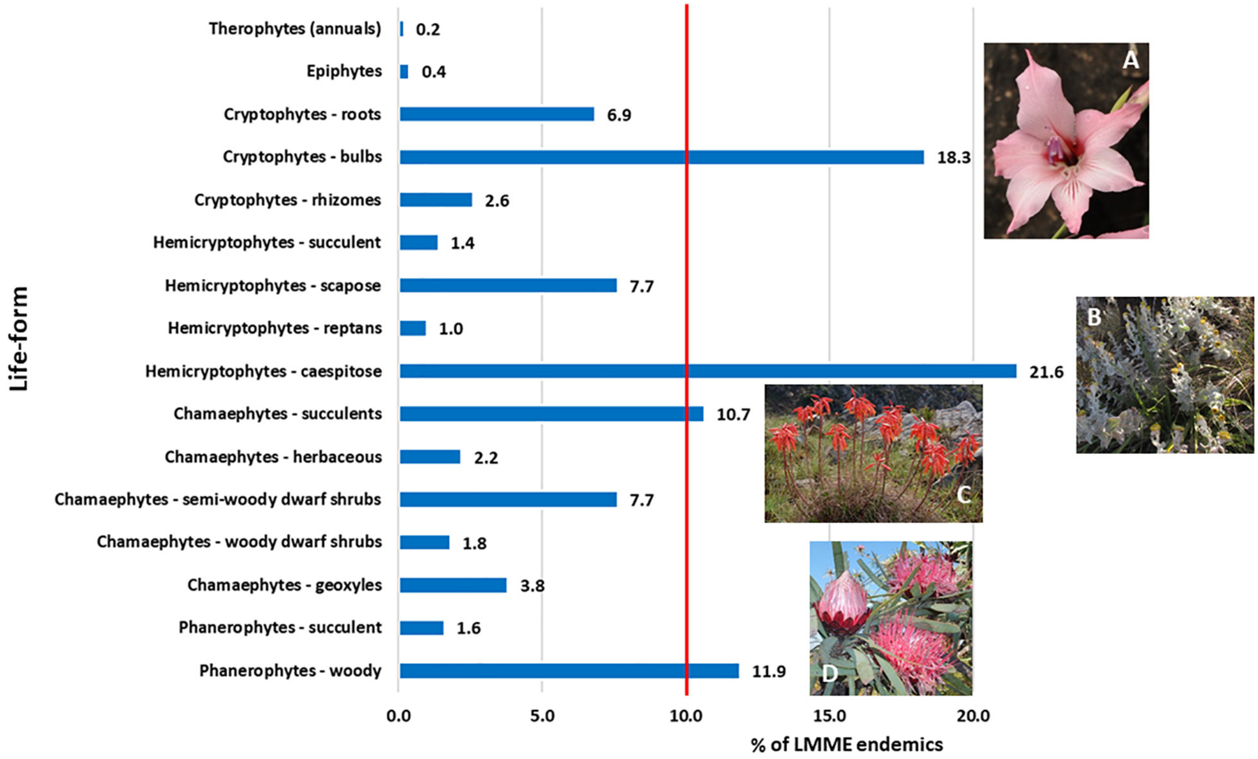

FIGURE 5 | Life-form partitioning among Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) endemic plant taxa. Life-form classes are based on a modified version of the Raunkiaer system (Raunkiaer, 1934); an additional category under chamaephytes was added, namely geoxyles: Geoxylic growth forms of woody subshrubs characterized by massive lignotubers, or underground woody axes, from which emerge aerial shoots which may be ephemeral given their misfit in the existing Raunkiaer system. **(A)** *Gladiolus pavonia* Goldblatt and J. C. Manning, an example of the abundant endemic bulbous cryptophytes in the LMEE; **(B)** *Helichrysum lesliei* Hilliard, a caespitose hemicryptophyte and an example of the dominant life-form endemic in the LMEE; **(C)** *Aloe chortolirioides* A. Berger is an example of a succulent chamaephyte in the LMEE; **(D)** woody phanerophytes such as *Protea curvata* N. E. Br. comprise a significant proportion of the LMEE endemic life-forms, many being endemic to the Grassland Biome. Credits: J. E. Burrows **(A)**, B. C. Turpin **(B)**, M. C. Lötter **(C)**, D. Oosthuizen **(D)**.

and capacity the Cape has. As a result, knowledge on absolute numbers of endemics—and enumeration thereof in the form of e.g., a Conspectus series—and a lack of the same level of sustained high research intensity, has limited an intimate knowledge of the drivers of speciation and patterns of endemism outside the Cape. The assumption has been that no-where else is as "special" floristically as the Cape, driving a positive feedback loop around ongoing intensive botanical work in the Cape. While the Cape region has the benefit of exceptional statistical power and analyses based on comprehensive plant distribution data, the extra-Cape region is somewhat behind on basic inventory and enumeration processes needed for more complex analyses. In the case of the LMEE, we provide the most comprehensive step toward rectifying this for one of the largest sections of Great Escarpment in the region, and certainly for what is probably the largest orographic entity in the summer rainfall region of southern Africa.

Various tallies of plant diversity and endemism in various parts of the LMEE have been published, mostly focusing on small sections of our LMEE. To date, Van Wyk and Smith's (2001) three Centers (Barberton, Wolkberg, Sekhukhuneland) provided the most comprehensive estimate of endemic diversity, with an estimated combined endemic suite of c. 310 taxa. Our exhaustive literature and data mining, synthesis of all novel taxa, and intimate knowledge of the LMEE add another c. 186 taxa to this, and upend earlier estimates of local endemism. While the LMEE is still not in the same league as the Cape (nor can it be compared until the montane component of the Cape Flora is extracted), it ranks as one of the most significant summer rainfall sections of Escarpment for local endemism, and consequent conservation priorities.

Different families and genera dominate in different biomes in the LMEE, which suggests phylogenetic partitioning of endemics between Biomes, possibly with different evolutionary lineages and drivers of speciation—although one cannot rule out Biome transgression in local speciation processes (e.g., for *Aloe* and *Helichrysum*). Our results support the primacy of open habitats (here dominated by Grassland) rather than closed habitats (e.g., Forest) in southern African mountains as the most important habitat for endemic taxa, supporting the earlier findings of Matthews et al. (1993) and Van Wyk and Smith (2001).

Life-Forms Among the Endemics

The dominant life-forms among the endemics highlight the dominance of the Grassland Biome in the LMEE. The rich non-graminoid endemic flora mirrors that of other primary grassland areas in southern Africa (Carbutt and Edwards,

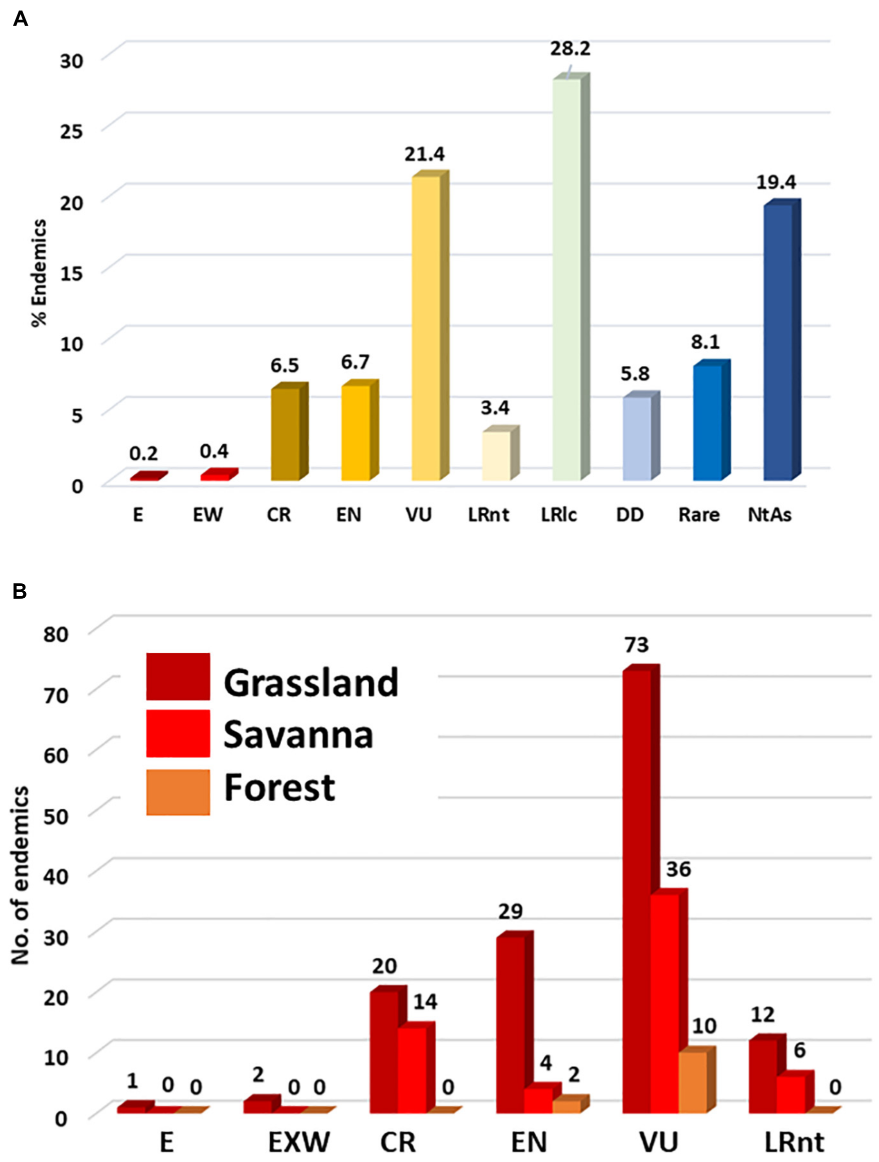

FIGURE 6 | Distribution of endemic plant taxa in the Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) according to **(A)** IUCN Red Data conservation status, and **(B)** according to those in the threat bundle (i.e., Lower Risk: Near Threatened to Extinct) per Biome. E, Extinct; EW, Exinct in the Wild; CR, Critically Endangered; VU, Vulnerable; LRnt, Lower Risk: near-threatened; LRlc, Lower Risk: least concern; DD, Data Deficient; NtAs, Not Assessed (Not Evaluated).

2006), and their irreplaceability for biodiversity conservation. Endemics were predominantly perennial herbaceous plants with seasonal shoot reduction due to frost, fire, or herbivory. Most of the endemics were expected to have this life-form, as the herbaceous flora of southern African grassy biomes is renowned for their resprouting ability in response to severe endogenous disturbances (Bombo et al., 2022). Noteworthy is that only four of these herbaceous endemics were graminoids and the rest were forbs.

Perennial herbaceous endemics were predominantly Cryptophytes and Hemicryptophytes. The largest group were Hemicryptophytes, which are characterized by a remnant shoot system with leaves that lie relatively flat on the ground. This group is globally the largest of the five major plant life-forms, and therefore this high endemism among Hemicryptophytes in the LMEE was predictable, as the group is well-adapted to open grassy ecosystems (Bond and Parr, 2010). Cryptophytes made up the second largest group of endemics—specifically geophytes that exhibit periodic reduction of the complete shoot system to storage organs that are imbedded in the soil and exhibit fire-stimulated flowering (Lamont and Downes, 2011). Bulbous geophytes are well represented throughout all the “open” Biomes of southern Africa, and are known for their endemism (Procheş et al., 2006); restricted range species would therefore be expected in the LMEE.

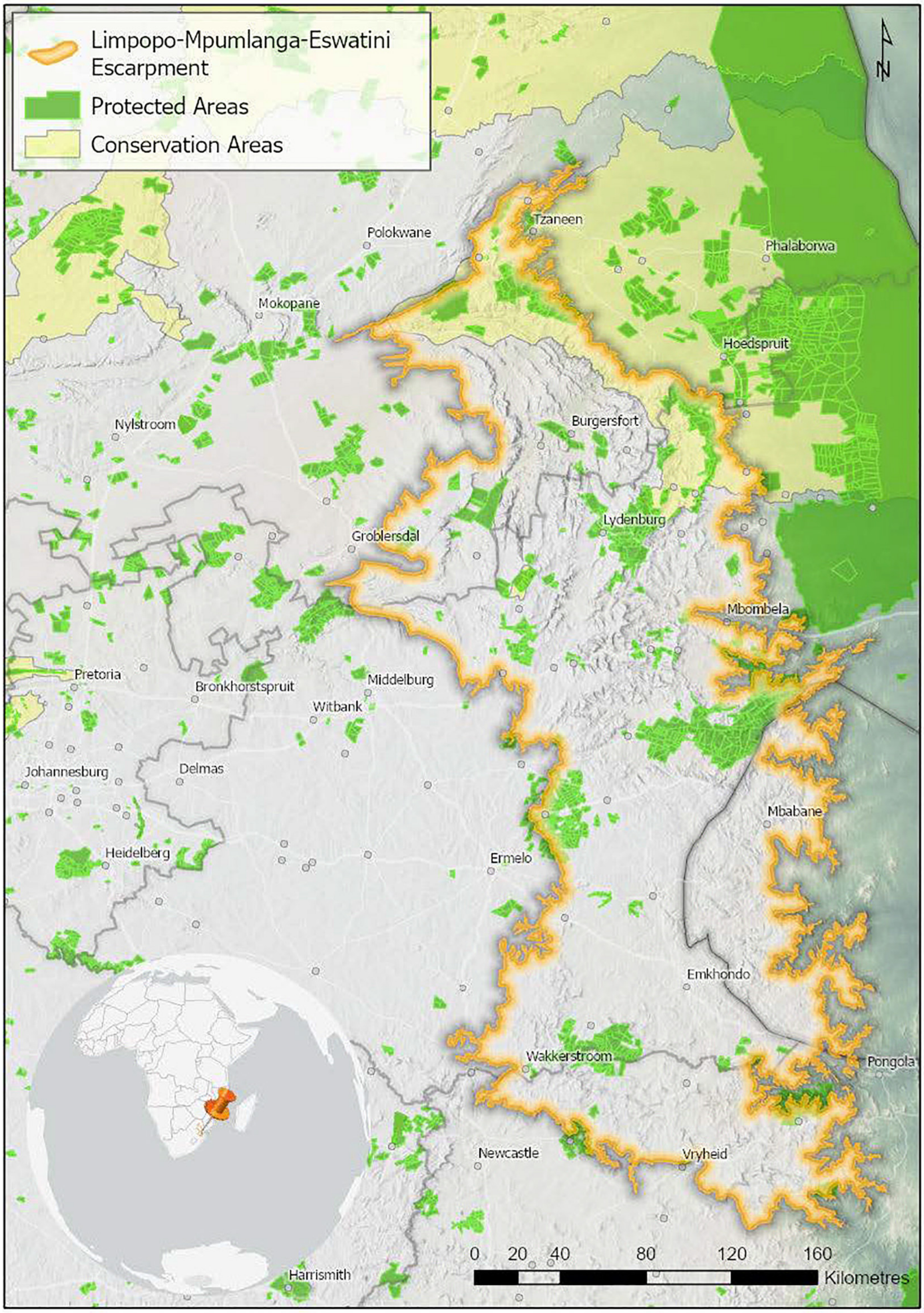

FIGURE 7 | Protected and conservation areas in the Limpopo–Mpumalanga–Eswatini Escarpment (LMME) in 2020. Protected areas are recognized in terms of the National Environmental Management Protected Areas Act (Act No. 57 of 2003), and restrict certain kinds of developments that may have a negative impact. Conservation Areas are more loosely defined, and offer little protection against a change in land use. Data from Department of Forestry, Fisheries and the Environment [DFFE] (2021a,b).

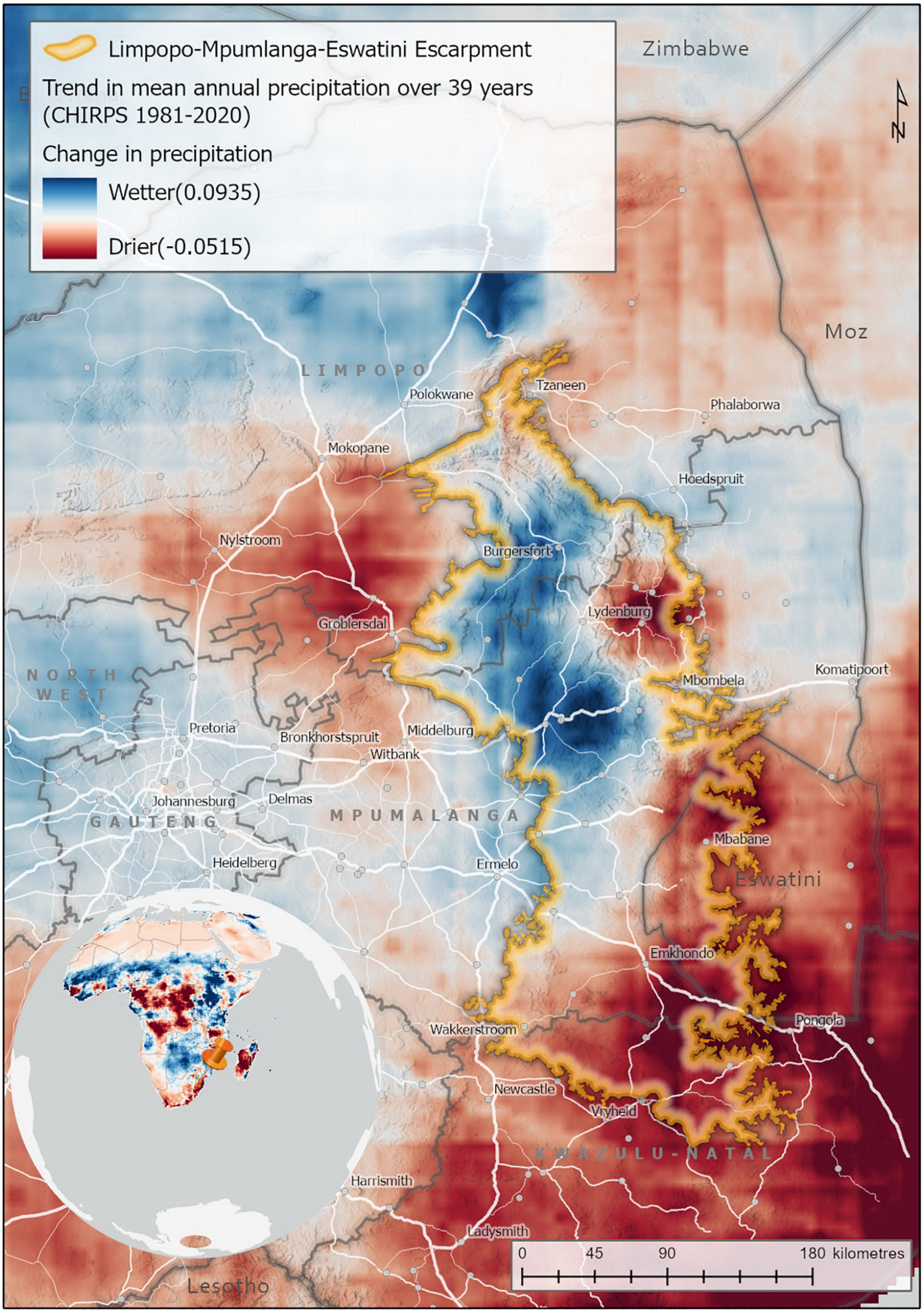

FIGURE 8 | Trends in mean annual precipitation in the Limpopo–Mpumalanga–Eswatini Escarpment (LMME) (1981–2020). Trend analysis estimates the trend or rate of change in mean annual precipitation for each pixel over a 39-year period using a linear trend function. The precipitation data sets used was the Climate Hazards Group InfraRed Precipitation with Station (CHIRPS) data, measured annually between 1981 and 2020 (Funk et al., 2015).

The well-represented endemic Phanerophytes comprise many species that are woody plants restricted to open grassy ecosystems rather than to forest (Matthews et al., 1993). These are fire-resistant/fire-tolerant small trees or shrubs (Hempson et al., 2014) that grow taller than 50 cm and have branches which do not die back periodically to that height limit, but which are often not more than 3 m tall in natural open habitat. These endemics are often scattered in the landscape and are typically associated with rocky outcrops and ridges (personal observations by the authors). The rich diversity of succulents in southern Africa also made a significant contribution to the LMEE endemic flora (Van Wyk and Smith, 2001): For example, endemic-richest genus *Aloe* are mostly succulent Chamaephytes that are prominent in both the Grassland and Savanna Biomes. Succulent Chamaephytes are tolerant of fire and frost and have mature shoot systems with succulent leaves that remain perennially 25–50 cm above the ground; even when growing taller, the shoots will die back periodically to that height limit due to endogenous disturbance (Pfab and Witkowski, 1999).

Distribution of Endemics Among Vegetation Types

Significantly, the exceptional endemism in the LMEE is driven by speciation in Grassland, most likely driven in response to herbivory, climate, and fire (Bond and Parr, 2010). This is significant—not because the value of Grassland for biodiversity is new knowledge in southern Africa—but because the fine-scale determination of endemism in an orographic context reveals (in the LMEE) to which extent this is true for southern African (montane) Grassland. In fact, ongoing fine-scale enumeration of endemism in the extra-Cape mountains may result in new areas of focal endemism that compete with the Cape for attention (such is the pattern emerging for the eastern Great Escarpment, of which the LMEE is the largest component). Similarly, the value of other open habitats (in this case Savanna) as a complementary endemic habitat to Grassland augment the value of the LMEE as an orographic entity. While Forest plays a less significant—but not unimportant (7.7% is a very significant proportion of endemics, considering the small area of Forest in the LMEE)—role in LMEE endemism, Forest in southern Africa typically hosts regional endemics rather than local endemics, and its value for endemism is at a regional rather than a local scale (Mucina et al., 2021).

Level of Conservation Concern

The most challenging conservation concerns in the LMEE are landscape transformation—especially conversion from mesic montane grassland to commercial forestry, i.e., plantations with alien timber (Kamffer, 2004); the rampant spread of alien invasive species; poaching of wild plant populations for horticulture and traditional uses (Emery et al., 2002); mining (particularly in Sekhukhuneland and in the south); and bush encroachment by indigenous woody species. Remaining Grassland in the LMEE should therefore be the primary focus of protection and conservation stewardship efforts (cf. Van Wyk and Smith, 2001).

Equally significant is that Grassland has not captured the public and policy imagination for conservation effort to the same extent as Forest (Van Wyk and Smith, 2001). As a result, open habitats in the LMEE are the least protected and the most transformed, resulting in a significant proportion of endemic taxa being at risk of extinction, with one species already Extinct and two Extinct in the Wild. Thus urgent focus on Grassland conservation is required in the LMEE. Coupled with this is the need for active trans-boundary co-operation with Eswatini. While there are numerous conservation areas "on paper" in the LMEE (i.e., the proportion of LMEE that is protected is encouraging), this is different to "effective conservation"—unfortunately many state-owned conservation areas are not receiving appropriate conservation management, as an example. Nor does it prevent continued harvesting of indigenous plants for various uses, including the extraction of cycads for the illegal market.

Future Research

While our treatment of LMEE endemics is considered the most comprehensive to date, the LMEE is rugged and difficult to access in many parts—notably the steep eastern scarp side; consequently—20 years after it was said by Van Wyk and Smith (2001)—there are still areas of the LMEE that remain unknown and warrant further careful exploration and biodiversity documentation (e.g., Von Staden, 2019 for the Thabakgolo Mountains). Such ongoing efforts are sure to yield additional novel taxa that will further increase the LMEE's pole-position as the endemic-richest extra-Cape montane system in southern Africa. Determining the actual spatial patterns of the endemic taxa in the LMEE, and how these patterns affect the distinctiveness of each Center, would be a next logical step: For example, Van Wyk and Smith (2001) postulated that merging these into one Center might be warranted on further study, with 3–4 sub-centers.

Drivers of this phenomenal endemism in the LMEE have been explored in part by Van Wyk and Smith (2001) but need careful teasing out. However, they are likely a combination of the following: extensive surface area, lower latitude (sub-tropical), high edaphic diversity, great topographic heterogeneity (despite not having particularly high elevations compared to the DMC and CME), relatively high botanical sampling intensity from sustained intensive botanical exploration over c. 150 years, steep climatic gradients from east to west and north to south, and the LMEE likely acting as both a refugium through climatic cycles (preserving and accumulating palaeo-taxa) and a species pump (of neo-taxa—likely driven by sister speciation on different edaphic substrates).

As indicated by Van Wyk and Smith (2001) for the Wolkberg Center, it may be that there also is a misalignment between concentrations of endemics/taxa of higher conservation concern and PAs throughout the LMEE. A valuable exercise would be to determine the overlap between LMEE endemic taxa distributions and the PAs/conservation areas.

CONCLUSION

The Limpopo–Mpumalanga–Eswatini Escarpment (LMEE) is a definable and discrete orographic component of southern Africa's Great Escarpment, and it is the largest component of the eastern Great Escarpment (i.e., between the Sneeuberg in the south and Nyanga in the north). With 496 endemic plant taxa, the LMEE has the highest plant endemism recorded for a mountain area in southern Africa to date—in terms of both absolute endemic numbers, and in terms of endemic richness (10.7%). The most similar montane system to the LMEE is the Maloti-Drakensberg (= Drakensberg Mountain Center), but the LMEE has almost double the number of endemic taxa, and a higher proportion endemism. In addition, botanical sampling intensity and intimate knowledge of the LMEE by the authors have played important roles in accurate and comprehensive listings of endemics, undoubtedly adding to the high tally. Grassland—and associated life-forms adapted to such open-habitats—is the most important habitat for endemics in the LMEE, from an evolutionary perspective and in terms of conservation concern.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

VRC provided the first draft of endemics, wrote the text, and created the figures. JB, BT, ML, KB, and SS contributed conceptual ideas, developed the full list of endemics (**Supplementary Material 3**), undertook data analyses and interpreted the data, and contributed to revising the manuscript. ML generated the maps. BT generated the **Supplementary Plates** and **Supplementary Table 1**. SS generated the tables. The manuscript concept was developed by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.765854/full#supplementary-material

Supplementary Material 1 | References used for determining plant taxa endemic to the LimpopoŰMpumalangaŰEswatini Escarpment (LMEE).

Supplementary Material 2 | Plant taxa endemic to the LimpopoŰMpumalangaŰEswatini Escarpment (LMEE).

Supplementary Material 3 | Endemic plant taxa richness for selected southern African mountains (summer rainfall zone); LMEE, LimpopoŰMpumalangaŰEswatini Escarpment. Data sources are provided in Table 2 of the main text.

Supplementary Material 4 | The families **(A)** and genera **(B)** with the highest number of endemics in each Biome in the LimpopoŰMpumalangaŰEswatini Escarpment (LMEE), ranked in order (top five are bolded). The number of LMEE endemics is given in parentheses.

Supplementary Material 5 | Land-cover change in the LimpopoŰMpumalangaŰEswatini Escarpment (LMEE) in a 30-year period (1990–2020). Percentages are of the total LMEE area.

Supplementary Plate 1 | A selection of landscapes in the Limpopo–Mpumalanga–Eswatini Escarpment (LMME): **(A)** Blyde River Canyon; **(B)** summit of Mariepskop (1,945 m); **(C)** Leolo Mountains, Sekhukhuneland; **(D)** Malolotja Game Reserve, Eswatini; **(E)** Mount Anderson, near Lydenburg; **(F)** Afro-temperate Forest, Buffelskloof Nature Reserve, Lydenburg; **(G)** eastern Songimvelo Game Reserve, Barberton Mountains. Photographs: S. J. Siebert **(A–D)**; M. C. Lötter **(E)**; J. E. Burrows **(F)**; K. Balkwill **(G)**.

Supplementary Plate 2 | A selection of plant endemics found in the Limpopo–Mpumalanga–Eswatini Escarpment (LMME), Part 1: **(A)** *Aloe chortolirioides* var. *woolliana*, Angle Station, Barberton; **(B)** *Brachystelma minor*, Hermansburg, Barberton; **(C)** *Brachystelma stellatum*, Morgenzon, Pilgrims Rest; **(D)** *Hesperantha brevicaulis*, Buffelskloof Nature Reserve, Lydenburg; **(E)** *Monopsis kowynensis*, God's Window; **(F)** *Ochna barbertonensis*, Bearded Man, Barberton. Photographs: M. C. Lötter **(A–C)**; J. E. Burrows **(D,F)**; B. C. Turpin **(E)**.

Supplementary Plate 3 | A selection of plant endemics found in the Limpopo–Mpumalanga–Eswatini Escarpment (LMME), Part 2: **(A)** *Pavetta glaucophylla*, Abel Erasmus Pass; **(B)** *Petopentia undulata*, Penge, Olifants River Valley; **(C)** *Tinnea barbata*, Barberton Mountainlands; **(D)** *Wurmbea burrowsii*, Mount Anderson, Lydenburg; **(E)** *Xerophyta purpurascens*, Buffelskloof Nature Reserve, Lydenburg. Photographs: J. E. Burrows **(A–C,E)**; K. Balkwill **(D)**.

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Bergmann's Rule Holds in Birds Inhabiting Southern Guinea Forests but Not in the Northern Savanna of Nigeria

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The Bergmann's rule predicts that in endotherms, body sizes will differ with respect to thermal gradients. Larger bodied individuals will inhabit colder environments while in warmer environments, individuals will be smaller-bodied. This hypothesis has been proved and disproved many times due to inconsistencies in body size differences along latitudinal gradients. We tested this hypothesis in 30 Afrotropical resident bird species inhabiting two vegetation types at different latitudes (southern guinea forests and northern savanna) and at different altitudes in Nigeria, West Africa. Using principal component analyses of body mass and wing length, the first principal component, the component of size, indicated that individuals in montane areas were larger than lowland populations in southern guinea forests. However, in the northern guinea savanna, there was no significant difference in body sizes between lowland and montane populations. General linear models show that body size increases as temperature decreases. In species found in both southern guinea forests and northern savanna (i.e., African Thrush *Turdus pelios* and Snowy-crowned Robin Chat *Cossypha niveicapilla*), variations in body sizes were significantly dependent on sites. Our study indicates that other macro-scale factors such as vegetation and rainfall patterns might modulate conformity to Bergmann's rule in Afrotropical environments.

Keywords: Bergmann's hypothesis, Afrotropics, thermal gradients, endotherms, altitude

INTRODUCTION

Bergmann's rule, an eco-evolutionary generalization predicting that endothermic animals will be larger in cold climates and smaller in warm climates, gives an explanation for the evolution of body size variation among similar groups of organisms (Bergmann, 1847; Salewski and Watt, 2017), which has been deployed to understand various aspects of ecology, such as predator-prey relationships (e.g., Mcnab, 1971). Although this hypothesis has been tested across different taxa, such as mammals (Clauss et al., 2013), birds (Ashton, 2002), and insects (Scriven et al., 2016), there is still an ongoing debate on its suitability for intra- and inter-specific studies (see Shelomi, 2012; Clauss et al., 2013). Simultaneous investigation of intra- and inter-specific variations in body size will enhance our understanding of the generalization of the Bergmann's rule. This is particularly important if such studies are conducted on little known species and regions such as Afrotropical regions.

The contrasting results obtained from the investigation of Bergmann's hypothesis suggest influence of other confounding factors, such as microclimates along latitudinal and altitudinal gradients (e.g., Shelomi, 2012; Bhusal et al., 2019). With the global climate change affecting all life forms on earth (Crozier et al., 2008; Şekercioĝlu et al., 2012), understanding species adaptation is important from an applied perspective. Species would need to adjust their physiology and behavior in order to cope with changing climatic conditions (Tieleman and Williams, 2000). Such studies are seldom reported from tropical areas, particularly Africa, which holds huge biodiversity, but projected to be adversely affected by global warming in the nearest future (Midgley and Bond, 2015; Sintayehu, 2018).

Due to their relative ease of trapping, birds are an important model for physiological studies. Here, we tested the Bergmann's rule within and across 30 avian species along vegetation and altitudinal differences in Nigeria, West Africa. While several studies have supported the Bergmann's rule, demonstrating that average body size within a species tends to increase toward cooler and higher latitudes (Ashton, 2002), we chose to test this rule along an altitudinal gradient because the north-south temperature gradient at the same elevation in Nigeria is small (an average difference of 7°C) and a previous study has already demonstrated a lack of relationship between latitude and body size in Nigeria (Nwaogu et al., 2018). We hypothesized a larger avian body size at high altitudes compared to lower altitudes and that the magnitude of size difference between lowland and montane populations will differ with respect to vegetation type (savanna vs. rainforest) and species.

MATERIALS AND METHODS

Study Sites

The data analyzed in this study were collected as part of the regular Bird Ringing activities of the A.P. Leventis Ornithological Research Institute (APLORI) located in northern Nigeria (Mwansat et al., 2011; Cresswell, 2018), on the Jos Plateau (9.5196°N, 8.5897°E; 1280 m asl) and at Yankari Game Reserve (9.7567°N, 10.5094°E; 330 m asl). In southern Nigeria, birds were trapped on the Obudu Plateau (6.3858°N, 9.3745°E; 1400 m asl) and at the lowland forests at International Institute of Tropical Agriculture (IITA; 7.2985°N, 3.5333°E) and Emerald Forest Reserve (EFR: 7.1780°N, 4.0806°E) both at 230 m asl (**Figure 1**). Because of close proximity (51 km apart), similar habitats and elevation, data from IITA and EFR were merged and analyzed as a single location (IITA). Data from Jos Plateau was from the period 200 to 2018, Yankari data was from 2011 to 2017, Obudu data was from the period 2005 to 2017, and the data from both IITA and Emerald was from the period 2017 to 2020.

The northern Nigeria sites are within the northern Guinea Savannah vegetation zone characterized by a mixture of trees and grass and receives annual rainfall of between 600 and 1,000 mm per mostly concentrated within 5–7 months (Ezealor, 2001; Omotoriogun et al., 2011; Braimoh et al., 2018). The southern sites are within the Guinea Forest vegetation zone characterized by dense evergreen forest of tall trees with thick undergrowth and receives annual rainfall of between 1,500 and 2,000 mm per annum with about 6–8 months of rainfall (Ezealor, 2001; Adeyanju et al., 2014; Awoyemi et al., 2020).

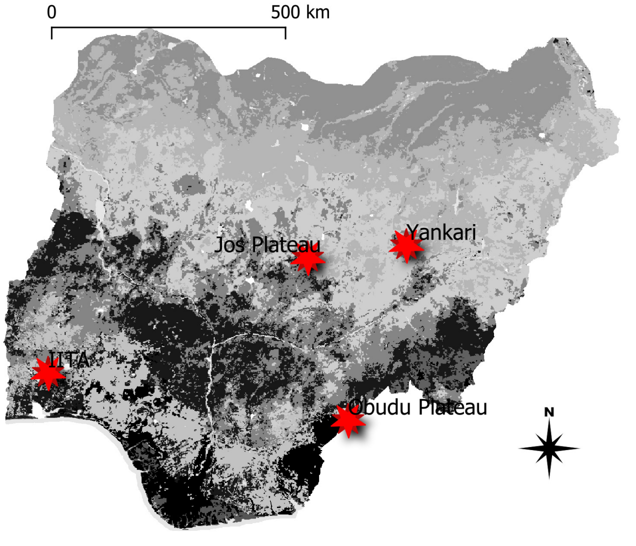

FIGURE 1 | Map of Nigeria with study sites indicated as stars.

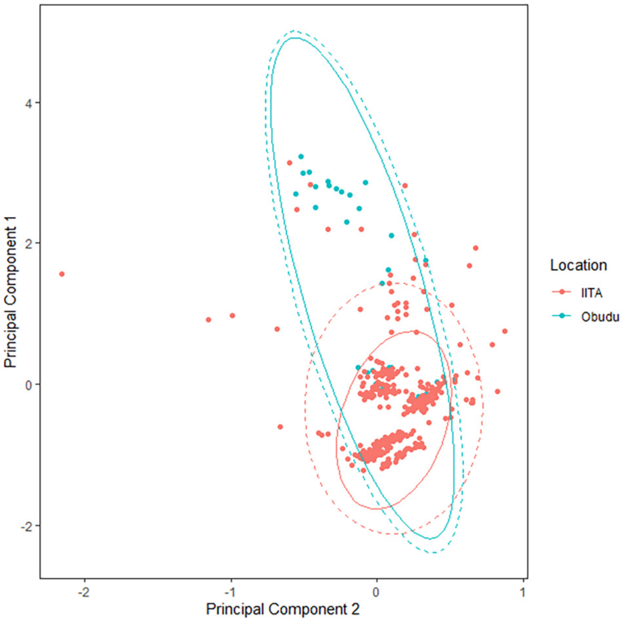

FIGURE 2 | Plots of principal component 1 vs. principal component 2 of lowland and montane species of southern guinea forest of Nigeria.

Data Collection and Analyses

Morphometric data of non-migratory bird species at low and high elevations at the northern and southern sites were used to test the Bergmann's rule. At all sites, birds were trapped with mist nets of various lengths at these different sites, tagged

TABLE 1 | General linear model investigating the relationship between body size (PC1) with sites (lowland vs. montane), species, temperature (model a), and rainfall (model b) in southern Nigeria.

		Parameter estimate	df	Sum of squares	*F*	*P*
Model a	Location		1	185.6	1664.1	<0.001
	Jos	0.25				
	Yankari	0.21				
	Species		5	559.0	1002.4	<0.001
	Temperature	–0.01	1	0.7	6.1	0.013
	Location: species		5	3.8	6.8	<0.001
	Location: temperature		1	0.0	0.3	0.609
	Species: temperature		5	2.3	4.1	0.001
	Location: species: temperature		5	1.6	2.8	0.017
Model b	Location		1	185.6	1591.5	<0.001
	Jos	0.22				
	Yankari	0.20				
	Species		5	559.0	958.7	<0.001
	Rainfall	–0.03	1	0.4	3.1	0.079
	Location: species		5	3.9	6.7	<0.001
	Location: rainfall		1	0.0	0.0	0.869
	Species: rainfall		5	1.0	1.8	0.118
	Location: species: rainfall		5	1.0	1.7	0.137

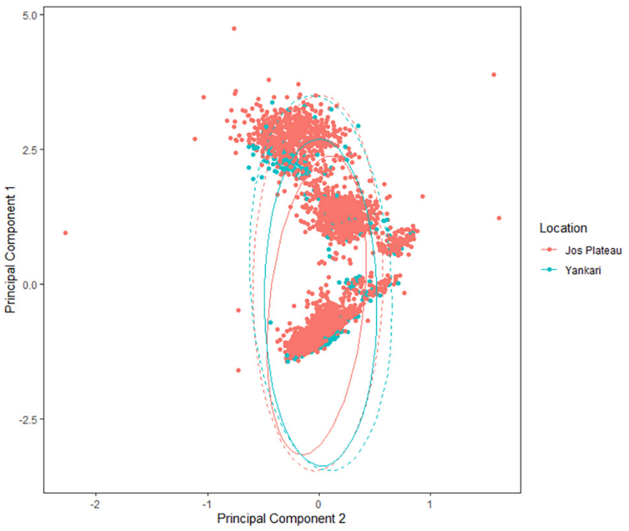

FIGURE 3 | Plots of principal component 1 vs. principal component 2 of lowland and montane species of northern savanna region of Nigeria.

with numbered metal rings, aged, sexed, weighed (g), and wing length (mm) measured. Only adult individuals of non-migratory or nomadic species were used for this study. Monthly precipitation and temperature data were obtained from the Bioclime website, [1] measured at 1 km^2 resolution, and extracted using the open-source program QGIS. These weather variables were used as covariates in further analyses.

For species with at least 10 records at both lowland and montane areas, we used the R package (R Core Team, 2016) to perform a principal component analysis (PCA) (Cozzolino et al., 2019) based on a correlation matrix using body mass and wing length. Both wing length and body mass are an indication of body size and have been consistently recorded at all ringing data but rather than analyze them separately, by using PCA, we can combine these two response variables into a single component to produce an index of body size. This procedure produces principal component scores for each individual; negative and low vales of scores indicate individuals with shorter wings and lighter body mass (i.e., smaller body sized individuals) while positive and higher values indicate larger body sized individuals. Because principal component scores had a Gaussian distribution, we used a general linear model to investigate if body size was affected by altitude (m), average monthly temperature (°C), total monthly rainfall (mm), using latitude as weighted variable. We used latitude as a weighting factor rather than an explanatory variable because a previous study testing the Bergmann's Rule along latitudinal gradient indicated that latitude had a slight but non-statistically significant effect on body size of birds in Nigeria (Nwaogu et al., 2018). By doing this, we also avoided over-parameterization of models while still adjusting for possible, if only slight, effect of latitude. To also avoided over-parameterization of models, the northern and southern sites were analyzed separately. Because temperature and rainfall were correlated, we analyzed their effect on body size in two separate models in order to avoid effects multicollinearity.

RESULTS

We analyzed data of six species in the southern Nigerian sites and 24 species in the northern Nigerian sites (**Supplementary Table 1**). The results of the principal component showed high and

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[1] http://www.worldclim.org/bioclim

TABLE 2 | General Linear Model investigating the relationship between body size (PC1) with sites (lowland vs. montane), species, temperature (model a), and rainfall (model b) in northern Nigeria.

		Parameter estimate	df	Sum of squares	*F*	*P*
Model a	Location		1	0.2	6.9	0.043
	IITA	0.21				
	Obudu	0.19				
	Temperature	–0.78	1	262.5	104.8	<0.001
	Species		25	9958.5	158.3	<0.001
	Location: temperature		1	0.1	5.4	0.019
	Location: species		23	2.3	4.1	<0.001
	Temperature: species		23	1.9	3.4	<0.001
	Location: temperature: species		23	1.0	1.7	0.021
Model b	Location					
	IITA	0.18				
	Obudu	0.20				
	Rainfall	–0.05	1	1.0	40.1	<0.001
	Species		25	10219.9	162.6	<0.001
	Location: rainfall		1	0.0	0.9	0.349
	Location: species		23	2.5	4.4	<0.001
	Rainfall: species		23	2.2	3.8	<0.001
	Location: rainfall: species		21	0.4	0.7	0.846

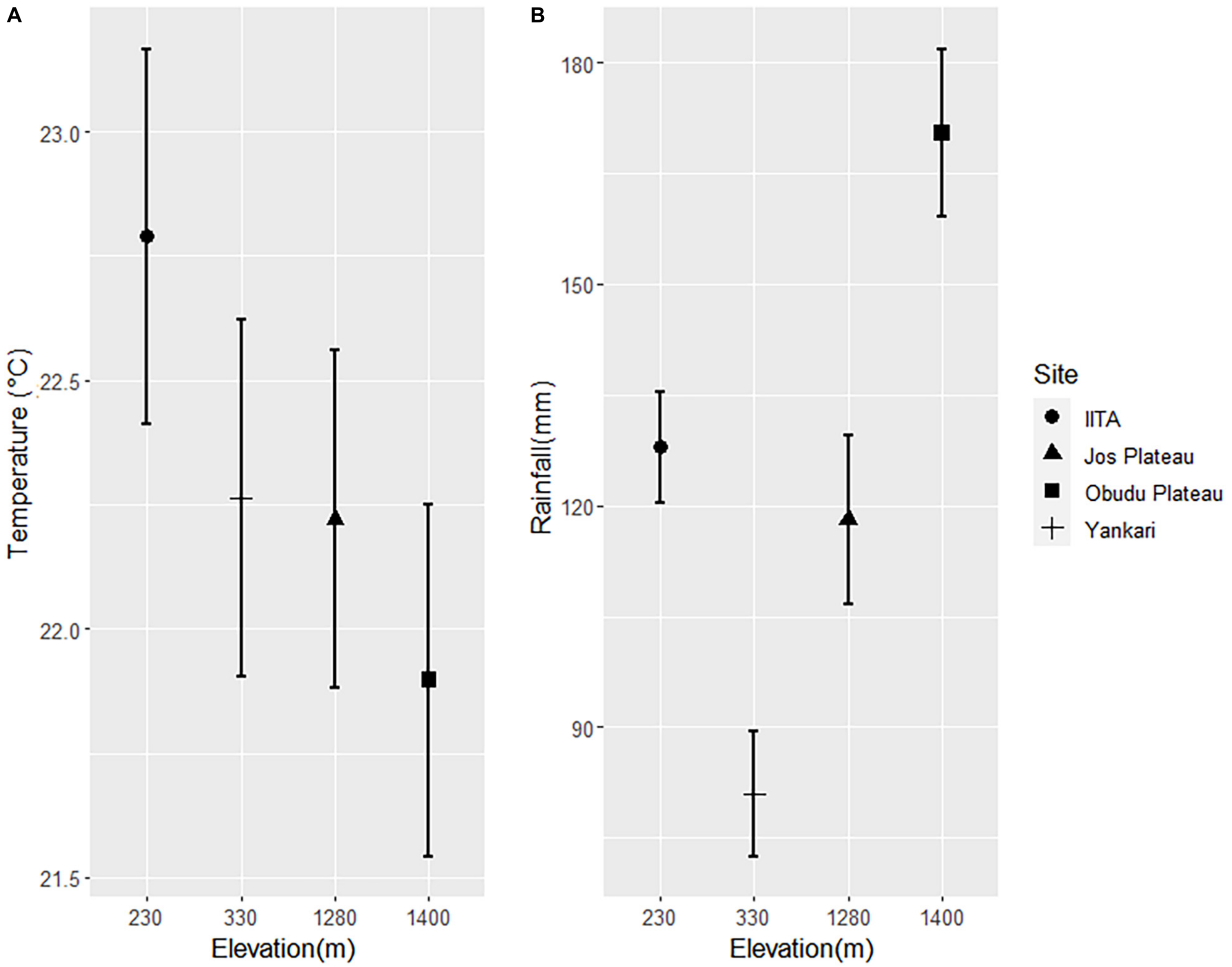

FIGURE 4 | Mean temperature **(A)** and rainfall **(B)** ranges across elevation gradients.

positive loadings on wing length and body mass (0.71 and 0.71, respectively) while PC2 had a high and negative loading on wing length (–0.60). Based on this result of the principal component analyses, Principal component 1 (PC1) represents the component of body size, accounted for 97.1% variability in the data while PC2 represents the component of wing length which made up 2.9% variability in the data.

A plot of PC1 against PC2 and the general linear model with site as predictor indicated that for the southern zone, montane populations were on average significantly heavier than lowland populations, but with lowland species having a comparable longer wing length (**Figure 2** and **Table 1**). Of the six species analyzed, only the Olive Sunbird *Cyanomitra olivacea* had a higher body size in the lowland population (**Supplementary Table 1**). However, since PCA 2 is the component of wing length and since lowland species have more positive scores along PCA 2 axis (**Figure 2**), it means that even though they are smaller than their montane counterparts, their wing length are comparatively longer. The GLM indicated that temperature was a significant predictor of body size, with body size and temperature having a negative relationship, The three-way interaction of location, species, and temperature was significant, indicating that differences in size between species was due to temperature variations between location (**Table 1**, Model a). Rainfall was not a significant predictor of body size and the three-way interaction between location, species, and rainfall was not significant either (**Table 1**, Model b), therefore, rainfall cannot explain the differences in body size between lowland and montane sites in the southern zone.

In northern sites, a plot of PC1 against PC2 showed a high degree of overlap in body sizes of lowland and montane populations (**Figure 3**), though montane populations were statistically heavier (**Table 2**). Of the 24 species considered in the study, populations of 17 species were heavier in montane areas while populations of seven species were heavier in lowland areas (**Supplementary Table 1**). A general linear model showed that both temperature and rainfall were significant predictors of body size (**Table 2**, Models a and b) with temperature showing a negative effect on body size. The three-way interaction of location, species, and temperature was significant indicating that temperature was responsible for the difference in body size of species at the different elevations. Although there was a significant effect of rainfall on the body sizes of species but the three-way interaction of location, species, and rainfall was not significant (**Table 2**, Model b). This is supported by weather data which shows that temperatures were lower at higher altitudes compared to lower (**Figure 4A**) latitudes whereas rainfall pattern did not follow and altitudinal trend (**Figure 4B**).

DISCUSSION

The findings of this study enhance our understanding of the validity of the Bergmann's rule, particularly from the understudied afrotropical region. In addition to determining the applicability of this rule among new afrotropical bird species (c.f. Nwaogu et al., 2018), our findings shed more light on the importance of microclimates while testing the Bergmann's rule along altitudinal gradients. To our understanding, this is the first study to simultaneously consider the interplay of these factors in the afrotropical context.

Although several studies have proven the effects of latitude on body size (see Olson et al., 2009; Shelomi, 2012; Clauss et al., 2013), we argue that these effects are dependent on habitat types. In the present study, montane populations were significantly heavier than lowland populations in the Guinea forest region. However, in the savanna region, there was extensive overlap in body sizes between montane and lowland populations. This pattern suggests the influence of other microclimates and macroclimate factors.

For the species that were found in both northern and southern zones, (African Thrush *Turdus pelios* and Snowy-crowned Robin Chat *Cossypha niveicapilla*), we used a *t*-test to also do a between-elevations comparison of body sizes across ecozones, i.e., body sizes of montane populations were compared between the north and southern zones and the same for lowland populations. Indices of body sizes indicated that populations in the southern zone were larger than the northern zone; body size on the Obudu Plateau were significantly larger than birds on the Jos Plateau (difference = 1.29, CI = 0.06–1.91, $p < 0.001$, **Figure 4**) and birds from IITA were also significantly larger than those of Yankari (difference = 0.36, CI = 0.12–0.59, $p < 0.001$; **Figure 5**).

For instance, we found that temperature significantly influenced body size in both rainforest and savanna regions, whereas, rainfall only had a significant impact on savanna species populations in the north. This is hardly surprising. Unlike temperature, the duration and volume of rainfall in Nigeria is significantly higher in the southern rainforests in comparison with the northern savanna (Anyadike, 1993; Obot et al., 2010). Shorter rainfall duration and intensity in the north would result in shorter periods of food availability,

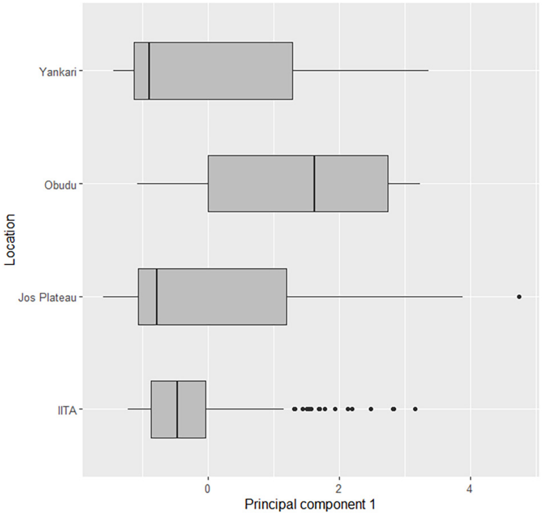

FIGURE 5 | Body size comparisons between northern and southern montane and lowland areas in Nigeria.

thus resulting in a lower body size index. In highly variable environments when food availability is limited or unpredictable, a larger body size is a disadvantage because larger bodied individuals require high energy intake in order to meet their energy requirement (Yom-Tov and Geffen, 2006). This premise is supported by our results which show that in the same species, the northern populations were smaller than the southern populations for both lowland and montane regions. In addition, it is likely that the temperature difference between the montane and the lowland habitat in the savanna region was not wide enough to impact on body size difference in the studied populations (**Figure 4**). Although there was a wide altitudinal gradient between the Jos Plateau and the lowland Yankari, the temperature difference between these sites was less than 1°C.

Our study has not only shown the applicability of the Bergmann's rule across afrotropical bird species, but also of its potential in understanding the impacts of climate change, which is important from a conservation standpoint. Although we attempted to fill knowledge gaps, our findings have exposed many others, which should provoke further studies. Since Africa supports a host of ecoregions and microclimates along latitudinal and altitudinal gradients (Olson et al., 2001), we would benefit more if further studies are conducted in understudied regions and species, particularly those that are sexually dimorphic (Blanckenhorn et al., 2006; Scriven et al., 2016). This study has also highlighted the need to study the potential impacts of climate change on avian species due to increasing temperature and erratic rains particularly its effect on the physiological state of organisms (Brown, 1996). Body condition, for instance, is known to influence survival and breeding in many species (Kitaysky et al., 1999; Souchay et al., 2013).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by SAFRING, South Africa and the A.P. Leventis Ornithological Research Institute, University of Jos.

AUTHOR CONTRIBUTIONS

YB and UO: conceptualization. YB: methodology and analyses. YB, AA, JA, and UO: validation and writing—original draft. UO: project supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.741755/full#supplementary-material

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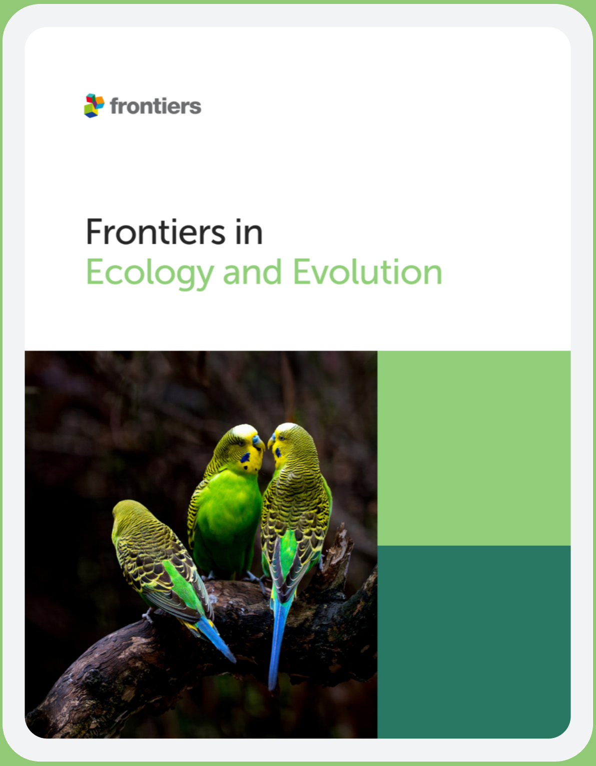

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